Impact of spectral quality of visible and UV light on the nutritional and the health-promoting qualities of leafy-vegetables and tomato

by

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B.S., Chungbuk National University, 2013
M.S., Chungbuk National University, 2015

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
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Abstract

Spectral quality of radiation has a major impact on the growth, development, and nutritional quality of crops. The solar radiation spectrum reaching the earth consists of both visible and UV regions, which have significant effects on plant growth and a myriad of plant functions. The main objective of this study was to examine the role of spectral quality of supplemental light (visible and UV) on the nutritional quality of lettuce and tomato with regard to their health-promoting phytochemicals and essential nutrients. The study included two lettuce varieties, (*Lactuca sativa* L. cv. red leaf 'New Red Fire' and green leaf 'Two Star') and tomato (*Solanum lycopersicum* L. cv. 'BHN-589') grown under growth chamber, greenhouse, and field conditions. The effect of supplemental visible radiation (blue, red, or far-red) on lettuce was determined under supplemental lighting provided by light emitting diodes (LEDs) in growth chambers with a background of white light (fluorescent lighting-PAR 270 μmol/m^2/s). Supplemental far-red produced higher biomass and larger leaf area in both lettuce varieties compared to the control plants. However, it had an inhibitory effect on the accumulation of many phenolic compounds in red leaf lettuce. On the one hand, in red lettuce, supplemental red radiation increased the accumulation of a number of phenolic compounds, while, on the other hand, supplemental blue radiation increased the accumulation of many phenolic compounds in green leaf lettuce. Thus, the results show that supplemental visible spectra of light can have a significant impact in enhancing the accumulation of health-promoting phytochemicals in lettuce. To determine the effect of supplemental UV on the nutritional quality in lettuce and tomato, the plants were grown under supplemental UV-A, UV-B, and UV-AB radiation using fluorescent lamps in a greenhouse. The overall response to these treatments was variable and was species and variety specific. UV-A produced a strong response in red leaf lettuce in improving the accumulation of many flavonoids. Higher accumulation of essential nutrients, including protein, phosphorus, calcium, magnesium, copper, zinc, and others, occurred in the green leaf lettuce in response to UV treatments. Supplemental UV-treatments of tomato plants, especially UV-B and UV-AB, produced higher accumulation of many phenolic compounds in mature fruits with no significant improvement in the essential nutrients. To further confirm the role of UV in enhancing the nutritional value of these crops, a field study was conducted where lettuce and tomato crops were grown in high tunnels covered with photo-selective poly covers to block solar UV radiation. Red leaf lettuce was the

most responsive to the UV containing treatment with regard to the accumulation of phenolic compounds. Clear poly and exposing the crop to full sun 2 weeks prior to harvest produced the largest increase in many flavonoids. Overall, the results suggest that the spectral quality of light, especially having the full spectrum of UV, plays a significant role in the accumulation phenolic compounds and essential nutrients and in improving the health-promoting qualities of both lettuce and tomato.

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most responsive to the UV containing treatment with regard to the accumulation of phenolic compounds. Clear poly and exposing the crop to full sun 2 weeks prior to harvest produced the largest increase in many flavonoids. Overall, the results suggest that the spectral quality of light, especially having the full spectrum of UV, plays a significant role in the accumulation phenolic compounds and essential nutrients and in improving the health-promoting qualities of both lettuce and tomato.

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Dedication

I dedicate this dissertation to my family, Jaekwang Lee, Duklee Jin, and Myungae Lee for their endless love, support, and encouragement. I would also like to dedicate this research to my God. He always gave me wisdom and strength to overcome challenging times.

Chapter 1 - Introduction

Solar radiation that reaches earth surface is the primary source of energy for life on earth, and a major part of this, approximately 95%, is in the visible spectrum (400-700 nm) while the rest is in the UV region (280-400 nm). A major part of UV is UV-A (320-400 nm), which accounts for 95% of the solar UV and the rest are UV-B (280-320 nm) and UV-C (100-280 nm) (Viršilė et al., 2019). Both visible and UV regions of solar radiation are essential for plant growth and development and play a critical role in a myriad plant functions. Plant responses to light are dependent on its spectral quality, intensity, and length of exposure (Sager J.C. and J.C. McFarlane, 1997). The visible spectrum such as blue, red, and far-red provides photosynthetically active radiation (PAR), which drives carbon accumulation in plants through photosynthesis, and it also plays a key role in biomass accumulation and many aspects of plant growth and development. Blue and red spectra are part of the PAR, and, in addition to providing energy for carbon assimilation in plants, they are involved in various plant functions including shoot elongation, shoot and root growth, cell differentiation, flower initiation, plant morphogenesis, and numerous physiological and metabolic processes including secondary metabolism (Hart, 1988; Hamm et al. 2004; Bian et al., 2014). Spectral quality of light can have a wide-ranging effect on the accumulation of secondary metabolites including several phenolic compounds and carotenoids (Bian et al.,2014). Many secondary metabolites are health-promoting phytochemicals, which are known to prevent many chronic diseases and, thus, promote health in humans (Prior and Cao, 2000).

There are a number of photoreceptors in plants that sense intensity, spectral quality, and duration of light exposure to facilitate numerous plant responses including plant morphogenesis (Viršilė et al 2017). Interactions of photoreceptors may be important in the photo-response of plants. For example, phytochromes, which are chromoproteins, can absorb red light and initiate a number of morphological and developmental changes in plants such as making a plant more shade tolerant, and many photoperiod dependent responses (Franklin and Whitelam, 2005; Mathews, 2006). However, these responses are blocked when phytochrome is exposed to far-red light or darkness. Since phytochrome can absorb both red and far-red light, plants have a unique ability to modulate their responses depending on the ambient spectral characteristics. Similarly, blue light, in addition to its important role in photosynthesis, can induce many morphological changes in plants including phototropism, apical dominance, and root growth (Briggs and Huala, 1999; Lin, 2002). The photoreceptors for blue light have been identified as phototropins and cryptochromes, which can also interact with UV to bring about a wide range of responses including light dependent gene expressions that can influence many physiological and metabolic processes in plants, including secondary metabolism (Lin, 2002;Yu et al. 2010).

Similar to visible radiation, UV radiation also plays an important role in plant growth and development. It influences photosynthetic activity, plant growth, biomass accumulation, plant morphogenesis, plant adaptation, and secondary metabolism (Teramura, 1983; Hollósy, 2002; Krizek, 2004; Verdaguer et al., 2017). Plants exposed to solar UV radiation accumulate a plethora of secondary metabolites including many phenolic compounds, especially flavonoids, which perform a defensive function in plants by absorbing damaging UV radiation, especially UV-B (Braun and Tevivni, 1993; Huché-Thélier et al., 2016). In addition, UV radiation is also known to induce the accumulation of many carotenoids, which are potent antioxidants. Similar to phenolic compounds, they are important health-promoting phytochemicals in our diet (Bian et al., 2016).

Despite the fact that a major part of solar UV is UV-A, which has a much stronger ability to penetrate plant tissues than UV-B, more attention has been devoted to examining the impact of UV-B in relation to the accumulation of phenolic compounds and plant growth. This is because

UV-B has higher energy and is more damaging especially at high doses than UV-A (Krizek, 2004). However, plant responses to UV is dependent on the complex interactions of UV-A and UV-B. In addition, UV radiation can also interact with ambient PAR in a way that can modulate the UV response. For example, high PAR levels can alleviate the typical negative impact of UV in plants, while lower PAR levels can produce just the opposite effect (Krizek, 2004).

With rapid advancement in light emitting diode (LED) technology in recent years, the use of LEDs has become increasingly popular as an alternate source of lighting for crops grown under protected environmental conditions such as greenhouses, growth chambers, high tunnels, and plant factories (Viršilė et al 2017). They are solid state devices and are popular because they are energy efficient, long lasting, and generate low heat compared to the conventional source of lighting. Another benefit of using LEDs is that it allows for the accurate control of the spectral quality of light, because they can be designed to provide output with a specific wavelength or in a narrow band of wavelengths (Folta et al., 2005). Thus, LEDs are ideal for characterizing plant responses in relation to specific quality of light. This allows for light factors, especially spectral qualities, to be used in manipulating many plant responses. In fact, LEDS with various spectral outputs are being used to improve crop growth and yield, control flowering, and enhance nutritional quality in many horticultural crops (Massa et al., 2008; Stutte, 2009; Meng et al., 2018). Many horticultural crops are increasingly grown under protected environmental conditions throughout the year using artificial sources of lighting provided by LEDs (Kospell et al., 2015). The most commonly used LEDs in protected environments are those with outputs in blue and red spectra, because they largely promote the photosynthetic function in plants (Son and Oh, 2013). However recently, UV-LEDs are also being used to improve the accumulation of secondary metabolites in many crop plants (Li and Kubota, 2009; Jeon et al., 2018).

Light and Phytochemicals and Essential Nutrients:

Abiotic and biotic stresses in plants can produce a number of reactive oxygen species (ROS) that are damaging to cells. As oxidative agents, they can oxidize a wide range of macromolecules including membrane lipids, carbohydrates, and nucleic acids (Lamb and Dixon, 1997). However, as a defensive mechanism, plants can produce numerous antioxidants to counter the adverse effects of ROS. The major antioxidants that can reduce the damaging effects of ROS in plants are secondary metabolites including numerous phenolic compounds and carotenoids, often referred to as phytochemicals. Interestingly, consumption of these phytochemicals can play an important role in suppressing the activity of ROS in humans as well. They are known to reduce the risk of several chronic and degenerative diseases such as heart disease, cancers, and neurological diseases including dementia (Steinmetz and Potter, 1996; Prior and Cao, 2000; Rajashekar et al., 2009). The primary source of phytochemicals in our diet is through plant-based food, notably fruits and vegetables. Thus, there is considerable evidence to indicate that consumption of fruits and vegetables, which are rich in these phytochemicals, reduces the risk of many diseases and, thus, promotes health (Prior and Cao, 2000). One of many external factors that can increase the accumulation of health-promoting phytochemicals in plants is abiotic stress. Previous studies have shown that, of all the various environmental stresses, high light intensity produces the strongest response in improving the phytochemical content of plants (Oh et al., 2009). This is further supported by many field studies, which show that crops grown in open fields have higher amounts of health-promoting phytochemicals and, thus, are nutritionally better compared to those grown in high tunnels under lower light intensity (Zhao et al., 2007: Oh et al., 2011). The crops grown in open fields have higher nutritional value because they have the benefit of receiving not only higher light intensity but also the substantial influence of UV-A and UV-B, while those grown in high tunnels or even in greenhouses are likely to receive low light intensity and much reduced levels of UV-A and UV-B. These studies clearly suggest that nutritional quality of crops can be substantially altered by manipulating light factors.

Fruits and vegetables, which are typically rich in health-promoting phytochemicals, are part of a healthy diet in reducing the risk of common diseases. They can improve the nutritional quality of our diet (Liu, 2013). However, the overall nutritional value of food comes from both its phytochemical and essential nutrient content. Consumption of a poor diet, which leads to malnutrition, is a serious global health issue. The most challenging problem globally is protein deficiency, which is more common and a serious challenge in the developing world (Müller and Krawinkel, 2005). However, the deficiency of mineral nutrients, like calcium, iron, and zinc, is widespread and is a critical challenge in most parts of the world including the U.S. (Ritchie and Roser, 2018). One of the practical approaches to mitigate this global health issue is to promote the consumption of a healthy diet containing nutrient-rich food. Therefore, improving nutritional quality in relation to both phytochemicals and essential nutrients is an important step in improving the overall quality of our food. Although there have been many studies aimed at understanding the role of light in improving the phytochemical content, very little is known about the role of quality of light in the accumulation of essential nutrients in plants.

In the present study, we investigate the role of spectral quality of light (both visible and UV) on the accumulation of health-promoting phytochemicals, such as phenolic compounds and carotenoids, in lettuce and tomato. These crops were chosen for this study because lettuce and tomato are the most popular vegetables, widely consumed, and some of the highest ranked fresh market vegetable crops based on their production and price value in the United States (National Agricultural Statistics Service, 2019). They are also a major source of health-promoting phytochemicals in our daily diet. In addition, they are the most commonly grown crops in protected environments where light factors can be easily controlled. In the present study, we controlled the spectral quality of light that these crops received in growth chambers and a greenhouse by using supplemental LEDs and conventional sources of lighting and by blocking selected spectral bands of solar radiation using photo-selective films in high tunnels under field conditions.

Thus, the broader objectives of this study were to understand the role of spectral quality of light on the nutritional quality of lettuce and tomato in relation to the accumulation of health-promoting phytochemicals and essential nutrients and to identify poly cover(s) that have the potential to enhance the nutritional quality of these crops in high-tunnel production.

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Chapter 2 - The Effect of Supplemental Blue, Red and Far-red Light on the Growth and the Nutritional Quality of Red and Green Leaf Lettuce

Abstract

Spectral quality of radiation has a major impact on the growth, development, and nutritional quality of crops. The effect of supplemental radiation (blue, red, and far-red) on the growth and nutritional quality with regard to health-promoting phytochemical and micronutrient composition of two lettuce (*Lactuca sativa*) varieties (red leaf 'New Red Fire' and green leaf 'Two Star') was studied. Supplemental radiation was provided by blue (450 nm), red (660 nm), or far-red (730 nm) LEDs against a background of white light (fluorescent lighting, PAR; 270 μmol/m^2/s) in a growth chamber study. All the supplemental radiation treatments increased dry shoot biomass in both varieties. Supplemental far-red radiation increased both fresh and dry shoot biomass in both varieties. In addition, supplemental far-red radiation produced distinct morphological characteristics in lettuce plants. It produced the largest shoot biomass, bigger and taller plants, fewer leaves but with larger leaf area compared to the control, similar to the shade avoidance response. With regard to the accumulation of phytochemicals, supplemental blue radiation enhanced the total phenolic compound concentration in both varieties. In addition, supplemental blue radiation increased the accumulation of several phenolic compounds in green leaf lettuce including chlorogenic acid, chicoric acid, rutin, kaempferol, luteolin, and apigenin. For example, the leaf concentration of rutin in green leaf lettuce increased by 20-fold under supplemental blue radiation. Similarly, supplemental red radiation increased the concentration many of these phenolic compounds in red leaf lettuce. However, supplemental far-red radiation had an inhibitory effect on the accumulation of chlorogenic acid, chicoric acid, rutin, and kaempferol in red leaf lettuce. While supplemental radiation did not affect the accumulation of most of the micronutrients, it had a negative impact on the accumulation of some micronutrients, the response being variety dependent. The results show that supplementing white light with specific spectral quality has a major impact on the biomass accumulation, morphology, and on the accumulation of many health-promoting phytochemicals and micronutrients in lettuce. While it had a large positive effect in enhancing the accumulation of several phytochemicals, it also suppressed the accumulation of some micronutrients.

Keywords: LEDs, lettuce, micronutrients, nutritional quality, phytochemicals, spectral quality

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2.1 Introduction

Light plays a vital role not only in many aspects of plant growth and development including seed germination, shoot and leaf growth, dormancy and flowering but also in the primary and secondary metabolism thus affecting the nutritional quality of plants [1-4]. Light intensity, spectral quality and duration of exposure to light (photoperiod) play a key role in photo-responses of plants. Manipulation of above light factors, especially spectral quality, provides a potential opportunity to enhance growth, control development and improve nutritional quality and aesthetic appeal of crops, which can enhance their marketability [5-10]. Photosynthetic function is among many factors that contribute directly to growth and biomass accumulation in plants, and blue and red radiation of the visible spectrum play an important direct role in this as they provide the energy needed for carbon assimilation. In addition to this, both blue and red radiation also regulate many aspects of morphogenesis including shoot elongation, cell differentiation, modulating shoot growth and flower initiation and also, numerous biochemical and physiological processes including those involved in secondary metabolism [8] [11]. Thus, notably spectral characteristics of light has a significant impact on the nutritional quality of plants including on the accumulation of health-promoting phytochemicals such as phenolic compounds, carotenoids and glucosinolates and micronutrients [7] [12-14].

Increasing number of food crops, especially horticultural food crops, are now being produced under environmentally controlled conditions using artificial source of lighting especially light emitting diodes (LEDs) [8]. The emerging LED technology has made it possible to use narrow band of light spectra to control growth, flowering, and nutritional quality of crops [3] [5] [6]. LEDs emitting red and blue radiations are commonly used to grow a number of crop plants in controlled environment conditions as they provide photosynthetically active radiation [15]. However, in recent years, green and far-red have also been added to the mix to modify plant growth and morphology in leafy vegetables [5] [16] [17].

The primary photoreceptors that are responsible for morphogenic responses in plants that allow the plants to sense and adapt to the quality, intensity and duration of light are red/far-red absorbing phytochromes and blue absorbing phototropins and cryptochromes [18] [19]. Phytochromes are chromoproteins containing 2 polypeptide subunits with a covalently bound tetrapyrrole (billin) chromophore sensitive to red and far-red light. There are two interconvertible forms of phytochromes, Pr and Pfr. Pr form absorbs red light and is converted into Pfr form which can be rapidly converted back to Pr form by absorbing far-red light. Interestingly, the absorption spectra of Pr and Pfr somewhat overlap and thus, there is a balance between these two forms of phytochromes. This helps the photoreceptor molecule to actually sense the quality of light that surrounds the plants, which in turn will allow for a greater control on the physiological processes and plant responses such as seed germination, photomorphogenesis, shade avoidance and photoperiod dependent responses [20]. The Pfr form of phytochrome produced in the presence of red light is considered as the physiologically active form, which can modulate many plant functions while far-red light can produce Pr form of phytochrome and has been linked to enhanced plant growth and shade avoidance strategies [21-23].

Blue light, in addition to its important role in photosynthesis, is also involved in morphogenic responses such as phototropism, suppression of stem elongation and stomatal regulation [19]. There are two types photoreceptors of blue radiation namely, phototropins and cryptochromes. Phototropins are flavoproteins which mediate phototropic responses in plants i.e., directional movement of plant organs toward light, control of stomatal opening and chloroplast movement. Cryptochromes are photolyase-like flavoproteins sensitive to blue light and are involved in photomorphogenic responses such as photoperiod controlled flowering, inhibition of hypocotyl elongation, stomatal opening, root development, apical dominance, light dependent gene expression and regulation of many physiological and biochemical processes [24] [25].

In a previous study, we found that environmental conditions have a notable impact on the secondary metabolism resulting in a significant accumulation many important health-promoting phytochemicals in lettuce [26]. High light intensity increased the accumulation of total phenolic compounds in the leaves by 3-fold compared to the control plants along with significant increases in chlorogenic acid, caffeic acid, chicoric acid, quercetin-3-O-glucoside and luteolin-7-O-glucodise. In addition, light elicits much stronger phytochemical response in lettuce than other environmental factors such as low or high temperatures. Previous studies have examined the spectral quality of light on plant growth and development and nutritional quality of plants including leafy vegetables using narrow-band LEDs. Often LEDs with different spectral outputs in various combinations (red, blue and green) have been used to study plant responses. This is an approach to identify the best combination of spectral quality that can produce the desired impact on plants [16]. However, this approach does not lend itself to discern effects of a specific spectral quality of light on plant functions. Therefore, in lieu of combination of spectral bands of radiation, in the current study we supplement traditional source of PAR (white light) with specific spectral quality in order to identify its impact on plant responses.

The objective of this study was to examine the impact of supplementing traditional white light (PAR) with red, blue or far-red radiation in order to evaluate the growth and the nutritional quality with regard to the accumulation of health-promoting phytochemicals and micronutrients in red and green leaf lettuce varieties.

2.2 Materials and Methods

2.2.1 Plant materials and growing conditions

Seeds of two varieties of lettuce *(Lactuca sativa*), a red leaf ‘New Red Fire’ and a green leaf ‘Two Star’ were sown in a commercial soil mix (Metromix 360, Sungro Horticulture, Agawam, MA) contained in seedling trays and the seedlings were grown for 2 weeks in a growth chamber (BDR16, Conviron Company, Winnipeg, Canada) set at 22°C (day/night) under fluorescent lamp (white light) with a photon flux of 300 μmol/m^2/s and a 12 h photoperiod. The seedlings were then transplanted into pots (12 cm X 12 cm X 12 cm) with the same soil mix as above and were subsequently transferred to a large growth chamber containing 3 open-top chambers (66 cm width X 56 cm depth X 77 cm height), each constructed out of white reflective particle boards. The chambers were used for supplementing the background white light (fluorescent lamps) with blue, red, or far-red radiation by using LED arrays. The growing conditions were 22°C (day/night) and 60% relative humidity with a photon flux for the background light (PAR) of 270 μmol/m^2/s and a 12 h photoperiod. The LED arrays consisted of LED bars (47 cm long) mounted vertically 16 cm apart along the walls of the open-top chambers. Each open-top chamber contained blue (peak 450 nm), red (peak 660 nm) or far-red (peak 730 nm) LEDs (Philips GreenPower Research Module; 24 V/ 10-15 W) and each LED bar had a photon flux output in the range of 13-16 μmol/s as supplemental source of lighting. The supplemental photon flux output in each open-top chamber was in the range of 78-96 μmol/s. Fluorescent background lighting without the LEDs represented the control.

Supplemental LED treatments were started when seedlings were 2 weeks old. Each LED treatment and the control had 4 replications and the experiment was laid out on a completely randomized design. Pots in each chamber were randomly rearranged in each open-top chamber every 2 days to minimize the uneven exposure of plants to supplemental radiation. Plants were watered every 2 days and fertilized once a week with irrigation water (N: P: K; 20:10:20) at 200 ppm of N.

All the growth characteristics were measured at the time of harvest (4 weeks after transplanting). The fresh biomass of shoots and roots was measured, and their dry biomass was obtained after drying them at 75^{o}C in an oven for 72 h. In addition, leaf number/plant was recorded. Leaf area was measured using a LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska). As the lettuce varieties used in this study are loose leaf type, the plant height was measured from the base of the plant to include the longest leaf in the canopy. Photosynthetic photon flux density (PPFD) was measured using a quantum radiometer (LI-185B, LI-Cor, Inc., Lincoln, NE). The PPFD measurements were made at the canopy level and the mean PPFD was obtained from 9 measurements made within each open-top chamber.

2.2.2 Chlorophyll and carotenoid measurements

Four lettuce plants from each treatment were randomly selected to determine the chlorophyll and carotenoid concentrations in their leaves. The freeze-dried leaf sample (0.3 g) was extracted with 3 mL 80% acetone for 25 min in an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT). The absorbance (A) of the extracts was read at 663 nm, 645 nm and 470 nm in a microplate reader (Synergy H1, BioTek, Winooski, VT). The leaf concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids were estimated according to the methods by Chen et al. [27] with some modifications using the following relationships:

$$\text{Chl a} = 12.72\ A663 - 2.59\ A645$$

$$\text{Chl b} = 22.88\ A645 - 4.567\ A663$$

$$\text{Total Chl a + b} = 20.3\ A645 + 7.22\ A663$$

Carotenoids = (1000 A470 - 3.27Chl a - 104Chl b)/229

2.2.3 Total phenolic compounds and antioxidant capacity

Total phenolic compounds were extracted according to Oh et al. [26] using the modified Folin- Ciocalteu reagent method [28]. Leaf sample (4 replications/treatment, 0.04 g each) was used to extract in 4 mL of 80% acetone using an ultrasonic processor (Vibra-Cell, Sonics and Materials, Inc., Danbury, CT) for 20 min and then 1.5 mL of the sample was transferred to a centrifuge tube and kept in the darkness overnight at 4°C. The extract was then centrifuged at 1000 rpm for 2 min and a 50 µL of the supernatant was mixed with 135 µL of distilled water, 750 µL diluted (1:10) Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 600 µL of 7.5% (w/v) Na_2CO_3. The mixture was vortexed and incubated in water bath at 45°C for 15 min and was then allowed to cool at room temperature. Absorbance was read at 765 nm (U-1100 Spectrophotometer, Hitachi Ltd. Japan). Gallic acid standards were made from freshly prepared gallic acid (Acros Organics, Belgium) in 80% acetone with 3 replicates for each concentration.

Total Antioxidant capacity was measured using ABTS decolorization assay as outlined by Miller and Rice-Evans [29] and Pennycooke et al. [30]. A 2.5 mM ABTS stock solution was prepared in 20 mL distilled water and the ABTS * radical cations were generated by adding 0.4 g of MnO_2 as an oxidizing agent to ABTS solution and stirring continuously at room temperature. Excess MnO_2 was removed by filtering under vacuum first, and then using 0.22 µm syringe end filter. Then the ABTS* solution was diluted to an absorbance value of 0.7 (±0.05) at 730 nm by using 5 mM PBS (phosphate buffer saline) at pH 7.4 and stored in water bath at 30°C. Trolox standards were prepared from a stock solution of 0.5 mM trolox. One mL of ABTS* reagent was added to trolox standards or samples and vortexed for 10 s and followed by 1 min of reaction time.

The absorbance of the reaction mixture was measured at 730 nm. A PBS solution was used as a blank for each assay. The antioxidant capacity of samples was estimated as the trolox equivalent.

2.2.4 Individual phenolic compounds and quantification

Freeze-dried and ground leaf samples (0.15 g) were extracted overnight with 15 mL of 70% aqueous methanol containing flavone as an internal standard on an orbital shaker (Benchmark, Edison, NJ) in a refrigerator at 4-degree. The extract was centrifuged and filtered 3 times to remove cell debris. Aqueous methanol was added to make up the final volume (25 mL) and 2 mL of this was evaporated to dryness under streaming nitrogen gas. The residue was re-dissolved in 1 mL of 70% methanol and then filtered through 0.22 μm syringe end filter (MilliporeSigma, Burlington, MA). The phenolic compounds were quantified using a Shimadzu HPLC system (Kyoto, Japan) consisting of a DGU-20A3 degasser, a LC-20AB liquid delivery pump, a SIL-20ACHT auto-sampler, a CTO-20AC column oven and a SPD-20A diode array detector. A C18 reversed phase column (250 mm L x 4.6 mm D, Waters, Milford, MA) was used to separate the phenolic compounds. The elution was conducted with a mobile phase A consisting of 5% formic acid in deionized water and mobile phase B consisting of 5% formic acids in 95% methanol at a rate of 0.8 mL/min and 31^{o}C oven temperature. The gradient used for solvent B was 0-10% for 5 min, 10-40% for 25 min, 40-70% for 10 min, and 70% for 16 min before returning to 0%. The phenolic compounds were separated and quantified using the method described by Woolley et al. [31]. The data were analyzed using the Shimadzu LC Solution Software (Kyoto, Japan). Concentrations of phenolic acids (gallic acid, chlorogenic acid, caffeic acid and chicoric acid) and flavonoids (luteolin, apigenin, kaempferol and rutin) were expressed as equivalents of vanillic acid and quercetin, respectively.

2.2.5 Essential nutrients

Leaf samples were collected at the time of harvest and were dried in an oven at 72°C for 72 h. The dried samples were ground in an electric grinder and the total carbon and nitrogen concentrations in the sample were determined using a LECO TrueSpec CN combustion analyzer. The concentrations of micronutrients including phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese and zinc were determined using an inductively coupled plasma (ICP) spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian, Australia PTY Ltd., Australia).

2.2.6 Statistical analyses

The mean separation of treatment effects was performed using Analysis of Variance (ANOVA) (SAS 9.4, Cary, NC and XLSTAT, Addinsoft, New York, NY) and the treatment comparisons were conducted using the Duncan's multiple range test for growth characteristics and micronutrient composition and by Tukey's test for phenolic compounds.

2.3 Results and Discussion

Red leaf (New Red Fire) and green leaf (Two Star) varieties of lettuce were grown in growth chambers with supplemental blue, red or far-red radiation using LEDs. Supplemental light of various spectral quality affected both shoot and root characteristics of both red and green leaf varieties of lettuce. However, the responses were variable and variety dependent. One common response of both varieties was that their exposure to far-red supplemental lighting significantly increased both fresh and dry biomass of shoots relative to the control plants (W) that received fluorescent lighting with no supplemental lighting (Figure 2.1). However, the largest increase in shoot fresh biomass in 'New Red Fire' was due to supplemental far-red radiation while such increase in 'Two Star' was with supplemental red radiation compared to the control plants. These increases in shoot fresh biomass were over 70% in ‘New Red Fire’ and over 56% in ‘Two Star’ compared to the control plants. While shoot fresh biomass increased with only supplemental far-red radiation in ‘New Red Fire’, it increased under both supplemental red and far-red radiation treatments in ‘Two Star’ relative to the control plants. However, all supplemental radiation treatments (blue, red and far-red) increased the dry biomass in both red leaf and green leaf varieties. Addition of red and blue radiation are expected to increase the biomass as both of these are directly involved in photosynthesis in converting the radiant energy into chemical energy to be used in fixing CO_2. Addition of far-red radiation may also aid in enhancing photosynthesis by perhaps increasing the plant leaf area, as we have found in this study (Figure 2.2). Supplemental far-red LEDs have been shown to increase fresh and dry weight, stem length and leaf area in red-leaf lettuce relative to white light [23]. With regard to the root growth, generally, ‘Two Star’ produced larger root system than did the ‘New Red Fire’ under control conditions (Table 2.1). Supplemental red radiation produced significant increases in fresh and dry root biomass in both ‘New Red Fire’ and ‘Two Star’, however, in addition to red radiation, supplemental blue radiation also enhanced fresh root mass in ‘Two Star’. The fresh root biomass was approximately 40 to 59% higher due to supplemental red radiation in both varieties than that in the control plants.

While far-red light increased the shoot biomass in both varieties, it had distinct effect on the morphology of lettuce plants. It significantly reduced the number of leaves while increasing the total leaf area in both varieties (Figure 2.2). Supplemental far-red radiation increased the leaf area by more than 45% in both varieties. Plants were larger under supplemental far-red radiation compared to the control plants. Under far-red supplemental radiation, plant height increased by approximately by 89% in the case of ‘New Red Fire’ and by more than 63% in ‘Two Star’ (Figure 2.2). High proportion of far-red radiation can trigger shade avoidance characteristics which include larger leaf area, elongated petiole, low chlorophyll content and fewer leaves [16] [32]. Plants receiving far-red will have higher phytochrome Pr form than Pfr resulting in shade avoidance syndrome which is characterized by changes in the morphological characteristics such as leaf area and plant height. This is often observed in crowded canopies which are exposed to greater amount far-red radiation (more Pr than Pfr) than direct red radiation, thus resulting in shade avoidance characteristics [21]. Also, in our study, there was much reduced leaf coloration in 'New Red Fire' under supplemental far-red radiation compared to the control plants while plants under supplemental blue produced deeper red foliage. The reduced coloration is due to lower leaf anthocyanin content when plants are exposed to far-red light [5] [23] while supplemental blue radiation has been known to increase the concentration of leaf anthocyanin content in lettuce [23]. Owens and Lopez [17] found that when red leaf varieties of lettuce were exposed to supplemental radiation of red, blue or a combination of equal ratio of red and blue for 5-7 days, there was an increase in their foliage color, which had a positive impact on their aesthetic appeal and marketability.

The response of total chlorophyll and carotenoids to specific spectra of light varied in lettuce varieties (Figure 2.3). In the red leaf 'New Red Fire', supplemental blue and far-red exposure increased both total chlorophyll and carotenoid concentrations in the leaves, in contrast both red and far-red exposure significantly reduced both total chlorophyll and carotenoid concentrations in the leaves of green leaf 'Two Star'. Thus, the response of chlorophyll and carotenoid contents in lettuce to spectral quality of light is dependent on the variety/genotype [8]. However, the response of total chlorophyll concentration in both varieties to supplemental radiation was similar to that of chlorophyll a while there were no significant changes in chlorophyll b concentrations to the supplemental radiation in both varieties, suggesting that chlorophyll a is sensitive to supplemental radiation rather than chlorophyll b (Table 2.2). In a study to examine the effect of supplemental LEDs on red leaf lettuce, Li and Kubota [23] found that supplementing white light with blue radiation increased xanthophylls and β-carotene in the leaves but not with red light while far-red light suppressed both of these carotenoids. Kopsell et al. [13] found that a combination of red and blue radiation from LEDs increased concentration of chlorophyll and carotenoids in the shoot tissue of broccoli microgreens grown under hydroponic system compared to the plants under conventional white light.

The total phenolic concentration and antioxidant capacity of leaves measured at the time of harvest (without any supplemental light treatment) were significantly higher in red leaf 'New Red Fire' compared to the green leaf 'Two Star' (Figure 2.4). This finding is supported by previous studies that show red leaf lettuce varieties have higher concentration of many health-promoting phenolic compounds than do green leaf lettuce [33] [34]. In our study, supplemental blue radiation significantly enhanced the concentration of total phenolic content and antioxidant capacity in the leaves of both varieties (Figure 2.4). However, the increases were more pronounced in green leaf 'Two Star' (nearly 75% over the control) compared to red leaf 'New Red Fire'. Similarly, studies by Stutte and Edney [35] on the effects of spectral quality of light on lettuce found an increase in antioxidant capacity by adding blue radiation to red radiation through LEDs.

We also examined the accumulation of individual phenolic compounds in the leaves of lettuce varieties as affected by supplemental radiation (Figure 2.5). Supplemental blue radiation in 'Two Star' significantly increased the accumulation of a number of phenolic compounds including chlorogenic acid, chicoric acid, rutin, kaempferol, luteolin and apigenin (Figure 2.5 and Table 2.3). It should be noted that the supplemental blue light resulted in a strikingly large accumulation of some of the phenolic compounds. For example, while leaves of control plants of 'Two Star' had very low concentration of rutin (26.82 μg/g DW), exposure of plants to supplemental blue radiation resulted in more than 20-fold increase in its concentration. Similarly, the supplemental blue light increased the accumulation of apigenin and kaempferol in leaves by more than 4.5-fold and luteolin by approximately 2.5-fold in the leaves of ‘Two Star’. Furthermore, exposure of ‘Two Star’ to supplemental far-red also had a positive impact in enhancing the accumulation of certain phenolic compounds such as chlorogenic acid, chicoric acid, luteolin and apigenin. Also, it should be noted that the increase in the accumulation of the many individual phenolic compounds in response to blue supplemental radiation in ‘Two Star’ is consistent with its higher total phenolic concentration in the leaves (Figure 2.4). Blue light has been shown to increase quercetin concentration and flavonol synthase, a key enzyme in the biosynthesis of quercetin, in both green leaf and red leaf varieties of lettuce [36]. In addition, blue light was also found to increase the accumulation of phenolic acids in basil and flavonoids in arugula [37]. Son and Oh [15] found that increasing blue radiation increased the total phenolic concentration, antioxidant capacity and total flavonoid concentration in both red and green leaf varieties grown under a combination red and blue LEDs.

In the case of ‘New Red Fire’, supplemental red radiation was most effective in enhancing the accumulation of phenolic compounds including chlorogenic acid, caffeic acid, chicoric acid, rutin, kaempferol, and luteolin. Similar observations of increased concentration of total phenolic compounds and antioxidant capacity were made in red leaf lettuce with supplemental red radiation [38]. While supplemental red radiation produced positive impact on the accumulation of many phenolic compounds in ‘New Red Fire’, supplemental far-red light drastically suppressed the accumulation of most of the phenolic compounds examined in this study. For example, the leaf concentrations of most of the phenolic compounds (caffeic acid, chlorogenic acid, chicoric acid, ruitn, kaempferol and luteolin) in ’New Red Fire’ grown under supplemental far-red light were much lower than those in the control plants and only ranged from 8 to 25% of the levels found in the control plants. The results show a strong inhibitory effect of far-red on the accumulation of phenolic compounds in ‘New Red Fire’ lettuce plants.

Leaves from plants grown under supplemental lighting were analyzed for carbon, nitrogen, and micronutrient composition at the time of harvest (Table 2.4). Generally, accumulation of most the nutrients was not affected by supplemental radiation treatments in both varieties. In the case of ‘New Red Fire’, the accumulation of most of the micronutrients did not significantly vary with respect to different supplemental radiation treatments except for manganese which accumulated in the leaves at much higher concentrations (about 1.4-fold higher) in plants subjected supplemental red light compared to control plants. Thus, in ‘New Red Fire’, supplemental red radiation had a positive impact not only on the accumulation of many phenolic compounds but also on the important micronutrient, manganese. However, in ‘Two Star,’ all the supplemental light treatments had a negative impact on the accumulation of nitrogen and zinc compared to the control plants. In addition, carbon accumulation was also suppressed by supplemental red and far-red radiation as well. It is not clear how the spectral quality of light may influence the accumulation of nutrients in plants. However, in a study on broccoli sprouts, Kopsell et al. [13] reported that the blue light exposure of plants increased the concentration of a number of micronutrients including manganese. They attributed this response to the influence of blue radiation on stomatal opening, membrane permeability and other membrane properties that may impact the transport of nutrients [8].

In summary, supplementing spectral quality of light has a major impact on the growth and nutritional quality of lettuce. Supplementing blue and red radiation produced large increases in many health-promoting phenolic compounds in green leaf and red leaf lettuce varieties, respectively. In addition, supplementing blue, red or far-red radiation resulted in higher shoot dry matter accumulation in both lettuce varieties. However, supplementing far-red radiation produced distinct morphological changes in both varieties such as enhanced shoot growth, increased leaf area and plant height but with reduced leaf number, similar to the shade avoidance response. Furthermore, it had an inhibitory effect on the accumulation of many phenolic compounds in red leaf variety. While supplemental radiation did not affect the accumulation of most micronutrients, it suppressed the accumulation of some micronutrients in lettuce.

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Figures and Tables

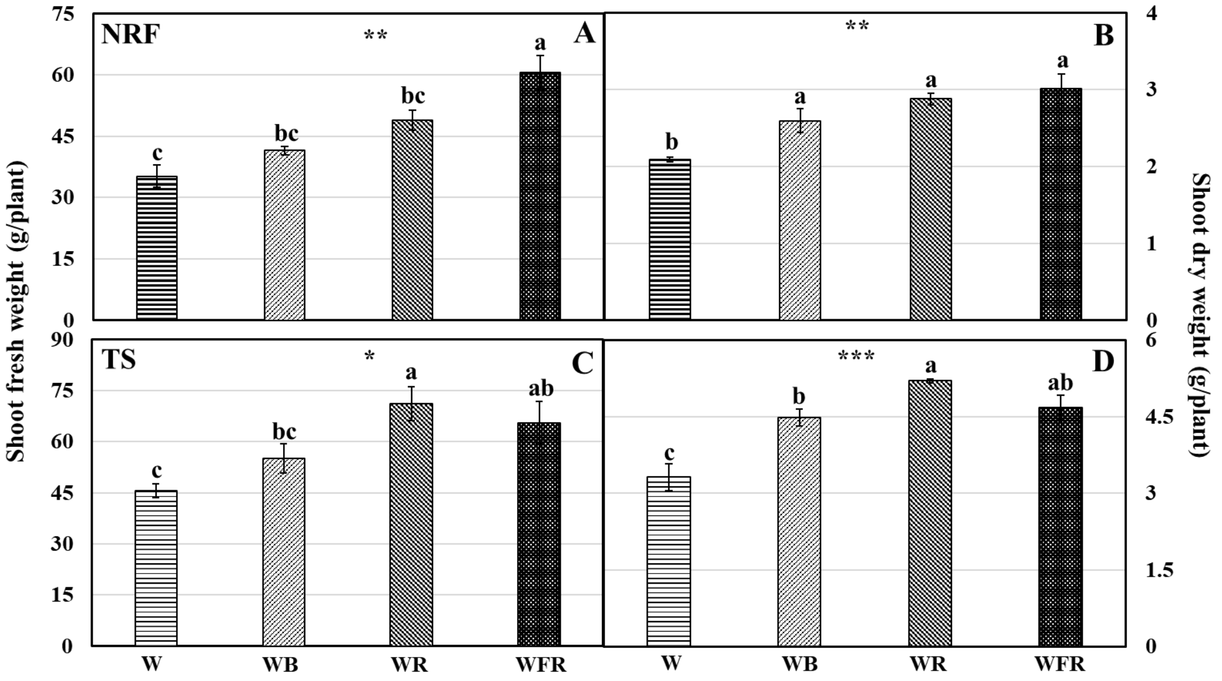

Figure 2. 1. Shoot fresh and dry weight in lettuce red leaf variety, ‘New Red Fire’ (NRF), and green leaf variety, ‘Two Star’ (TS), and supplemental LED radiation. Fresh weight (A) and dry weight of shoots (B) for NRF and fresh weight (C) and dry weight of shoots (D) for TS in response to fluorescent white light (W-control), supplemental blue (WB), red (WR) and far-red (WFR) are presented. Vertical bars indicate SE and significant differences are indicated at $p < 0.05$, $p < 0.01$ and $p < 0.001$ with *, ** and ***, respectively.

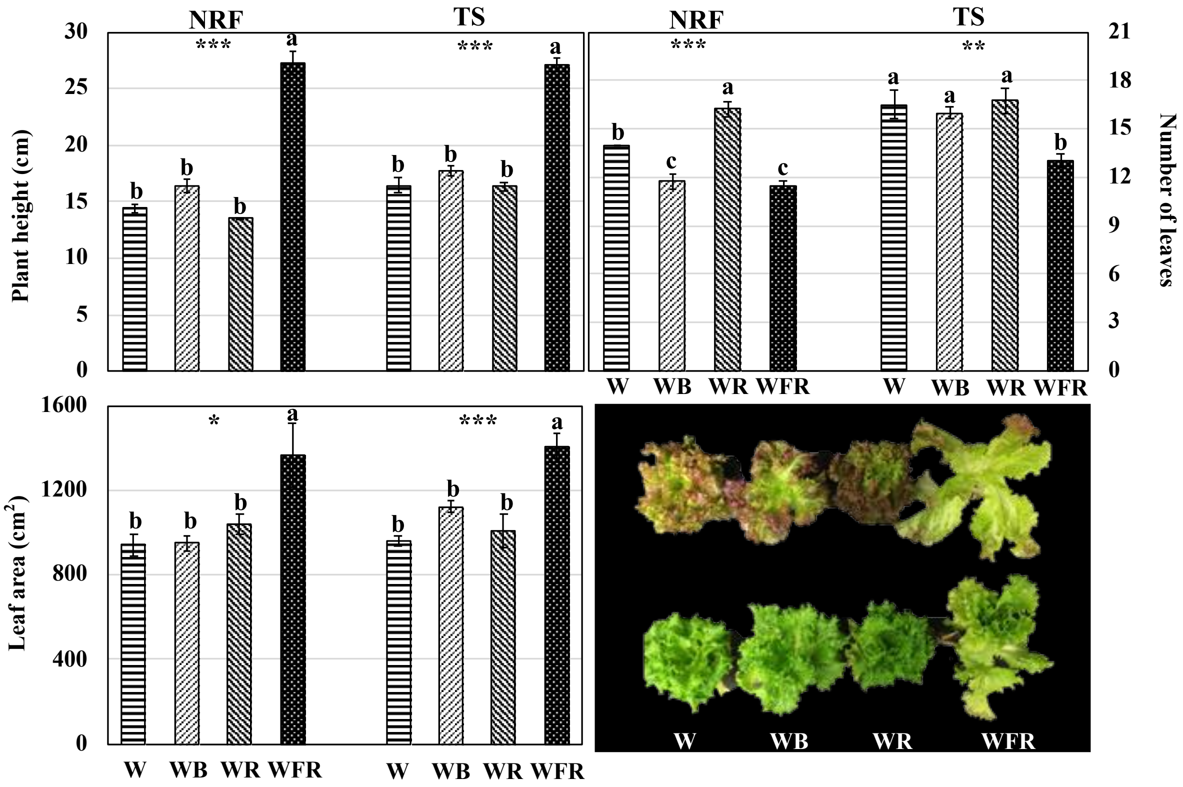

Figure 2. 2 Growth characteristics of lettuce red leaf variety, 'New Red Fire' (NRF), and green leaf variety, 'Two Star' (TS) and supplemental LED radiation. Plant height, number of leaves/plant and leaf area in response to fluorescent white light (W-control), supplemental blue (WB), red (WR) and far-red (WFR) are presented. Photograph shows plants subjected to various light treatments (top row- 'New Red Fire' and bottom row- 'Two star'). Vertical bars indicate SE and significant differences are indicated at $p \leq 0. 05$, $p \leq 0.01$ and $p \leq 0.001$ with *, ** and ***, respectively.

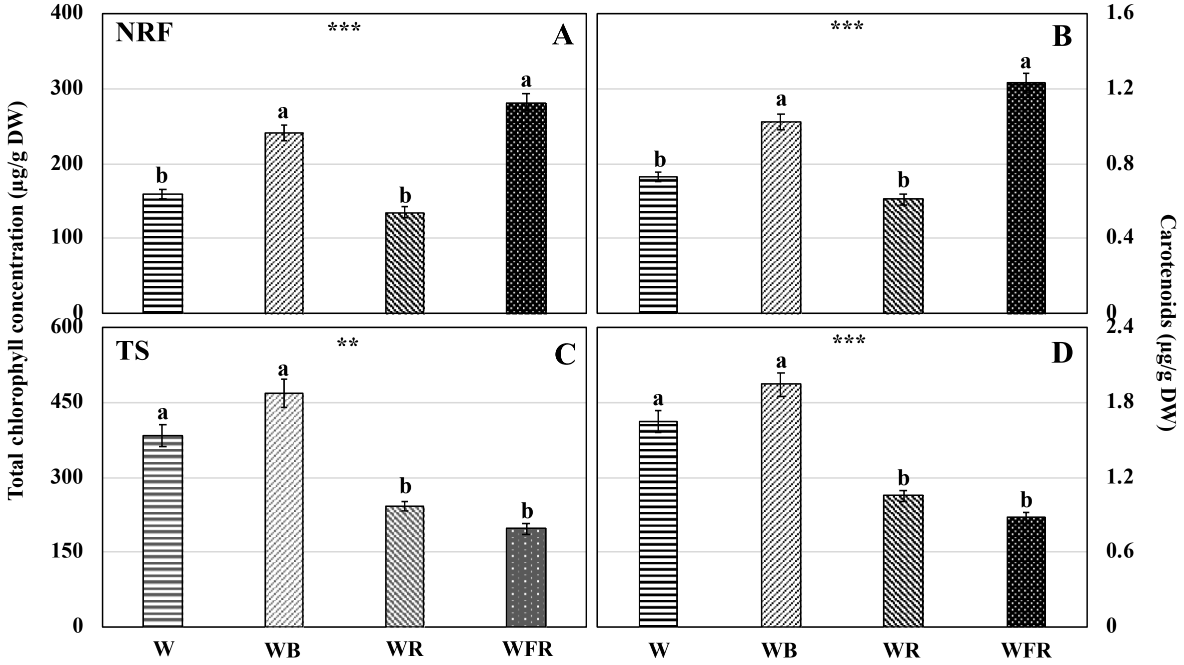

Figure 2. 3 Chlorophyll and carotenoid concentrations in leaves of lettuce red leaf variety, 'New Red Fire' (NRF), and green leaf variety, 'Two Star' (TS), and supplemental LED radiation. The treatments included fluorescent white light (W-control), supplemental blue (WB), red (WR) and far-red (WFR). Vertical bars indicate SE and significant differences are indicated at $p < 0.01$ and $p < 0.001$ with ** and ***, respectively**.**

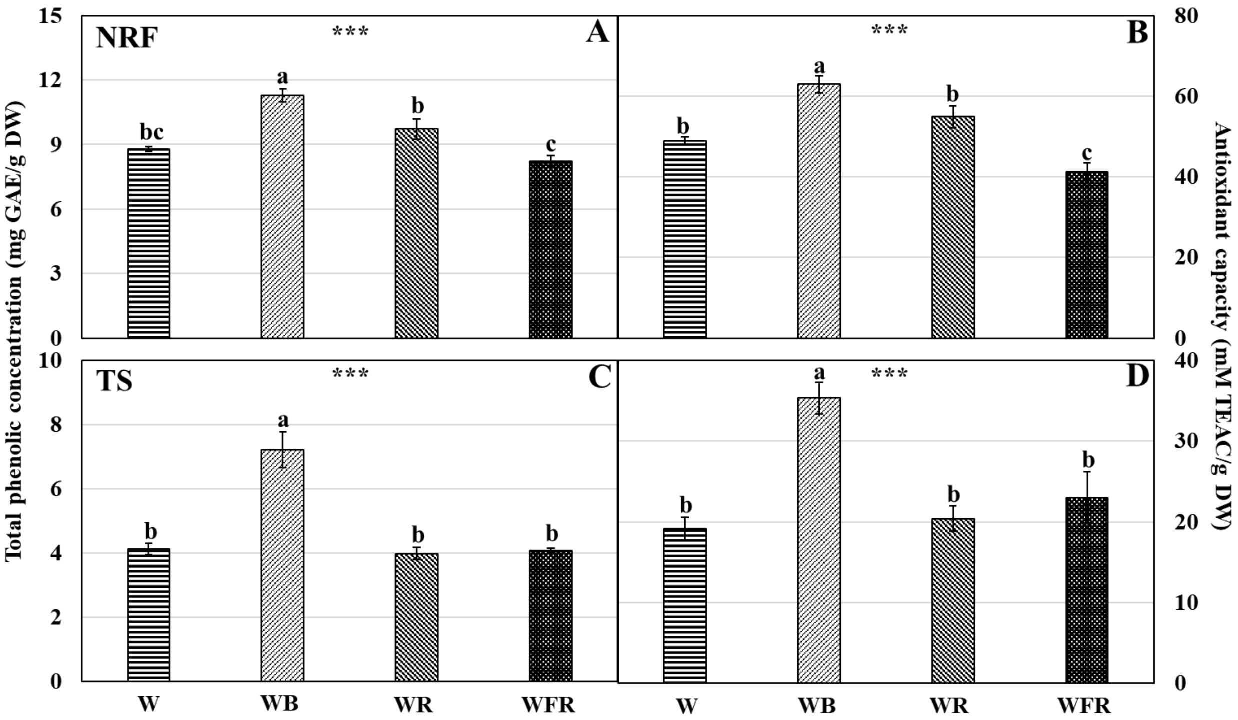

Figure 2. 4 Total phenolic concentration and antioxidant capacity of lettuce red leaf variety, ‘New Red Fire’ (NRF), and green leaf variety, ‘Two Star’ (TS), and supplemental LED radiation. Total phenolic concentration and antioxidant capacity in NRF (A and B, respectively) and in TS (C and D, respectively) are shown. The treatments included fluorescent white light (W-control), supplemental blue (WB), red (WR) and far-red (WFR). Vertical bars indicate SE and significant differences are indicated at $p < 0.001$ with ***.

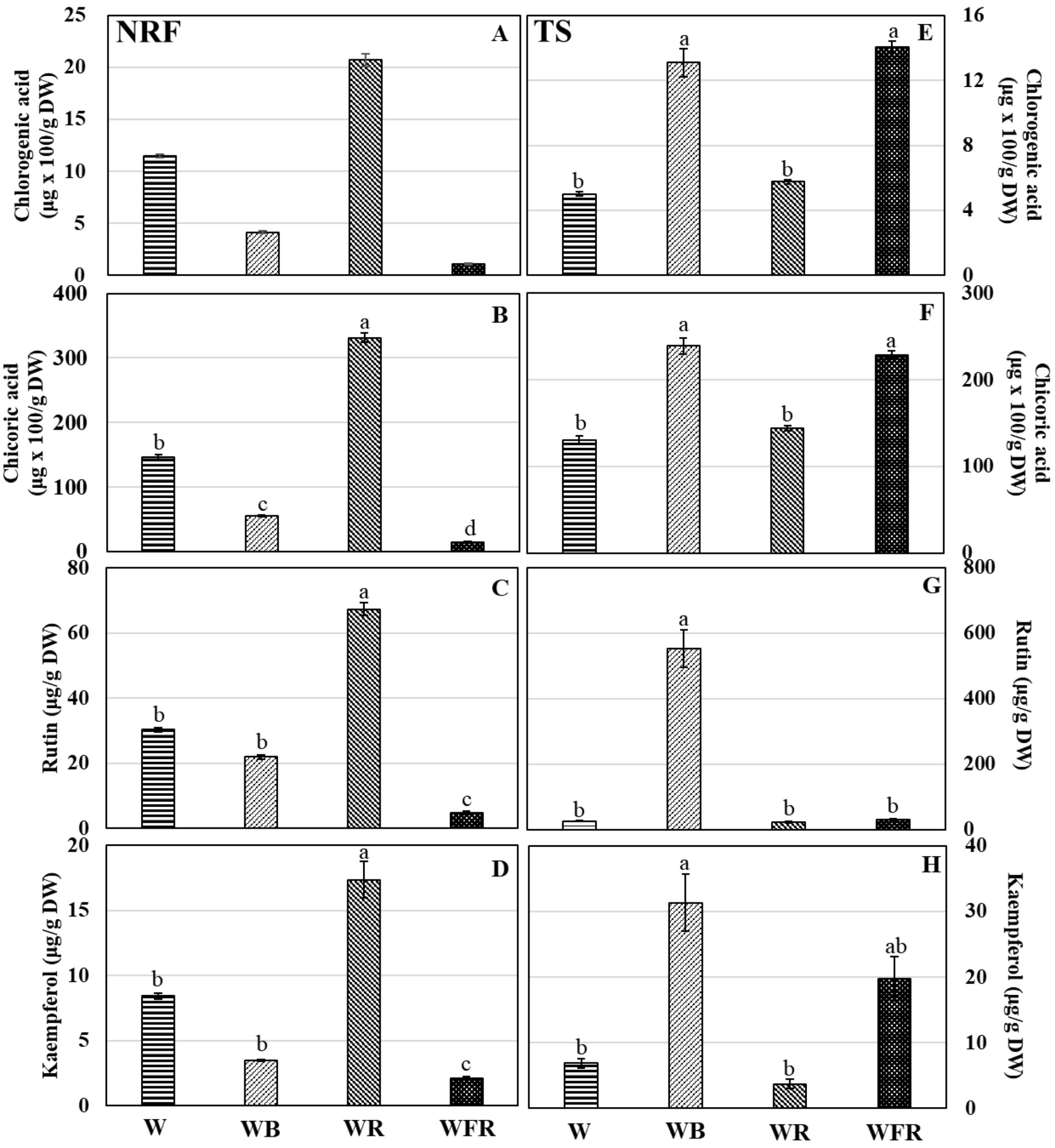

Figure 2. 5 Concentration of phenolic compounds in lettuce red leaf variety, ‘New Red Fire’ (NRF-A, B, C and D), and green leaf variety, ‘Two Star’ (TS-E, F, G and H), and supplemental LED radiation. The treatments included fluorescent white light (W-control), supplemental blue (WB), red (WR) and far-red (WFR). Vertical bars indicate SE and significant differences are indicated by letters at $p < 0.05$.

Table 2. 1 Root fresh weight (FW) and dry weight (DW) in red leaf 'New Red Fire' and green leaf 'Two Star' lettuce in response to supplemental blue, red and far-red radiation. Supplemental radiation was provided by LEDs against a background of white light (W).

	New Red Fire		Two Star	
	Root		Root	
	FW (g/plant)	DW (g/plant)	FW (g/plant)	DW (g/plant)
W	9197.03 b	677.50 b	12285.60 b	1023.13 b
W-Blue	8324.47 b	548.47 b	18131.23 a	1568.67 ab
W-Red	13007.47 a	1021.97 a	19590.13 a	1912.43 a
W-Far-red	8049.63 b	596.43 b	11511.50 b	940.87 b
Significance	***	***	**	*

Significant differences are indicated by letters at $p < 0.05$, $p < 0.01$ and $p < 0.001$ with *, ** and ***, respectively.

Table 2. 2 Chlorophyll a and chlorophyll b concentration in leaves of red leaf 'New Red Fire' and green leaf 'Two Star' lettuce in response to supplemental blue, red and far-red radiation. Supplemental radiation was provided by LEDs against a background of white light (W).

	New Red Fire		Two Star	
	Chl a (μg/g DW)	Chl b (μg/g DW)	Chl a (μg/g DW)	Chl b (μg/g DW)
W	121.3 b	4.35	298.6 a	4.17
W-Blue	188.2 a	4.16	363.7 a	4.21
W-Red	104.6 b	4.22	187.6 b	4.24
W-Far-red	217.7 a	4.29	152.2 b	4.24
Significance	***	NS	**	NS

Significant differences are indicated by letters at $p < 0.01$ and $p < 0.001$ with ** and ***, respectively. NS represents no significant differences.

Table 2. 3 Phytochemical concentration in leaves of red leaf ‘New Red Fire’ and green leaf ‘Two Star’ lettuce in response to supplemental blue, red and far-red radiation. Supplemental radiation was provided by LEDs against a background of white light (W).

		Phytochemicals (µg/g DW)			
		Gallic acid	Caffeic acid	Luteolin	Apigenin
New Red Fire	W	85.05±8.3^{ab}	48.67±2.7^{b}	259.70±11.5^{b}	-
	W-Blue	4.63±0.5^{b}	13.40±9.0^{c}	145.62±14.2^{c}	-
	W-Red	101.50±64.0^{a}	218.74±15.5^{a}	405.07±74.1^{a}	-
	W-Far-red	5.13±1.8^{b}	4.37±1.4^{d}	24.89±6.4^{d}	-
Two Star	W	18.75±11.1^{ab}	100.93±13.7^{ab}	172.91±14.9^{c}	3.92±0.04^{b}
	W-Blue	27.70±16.9^{ab}	116.97±45.2^{ab}	442.28±28.1^{b}	19.47±4.0^{a}
	W-Red	6.33±4.6^{b}	59.08±35.0^{b}	228.40±56.9^{c}	1.86±0.1^{b}
	W-Far-red	54.48±21.2^{a}	178.4±36.9^{a}	686.43±70.7^{a}	17.91±7.6^{a}

The values are presented with SD and significant differences are indicated by letters at $p < 0.05$.

Table 2. 4 Leaf concentration of nutrients in red leaf 'New Red Fire' and green leaf 'Two Star' lettuce in response to supplemental blue, red and far-red radiation. Supplemental radiation was provided by LEDs against a background of white light (W)

	Light source	N (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO4-S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
New Red Fire	W	2.718	36.42	0.559	4.87	0.794	0.316	0.204	5.10	109.58	89.78b	49.48
	W-Blue	2.565	36.88	0.566	4.64	0.783	0.324	0.245	4.95	104.53	96.43b	49.65
	W-Red	2.388	36.14	0.553	4.80	0.933	0.348	0.224	5.15	165.38	129.08a	52.35
	W-Far-red	2.842	35.90	0.571	4.94	0.821	0.364	0.267	4.65	165.45	80.43b	43.10
	Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Two star	W	2.073a	38.68a	0.398	4.02	0.925	0.255	0.196	3.78	80.60	92.58	34.73a
	W-Blue	1.718b	39.07a	0.359	3.62	0.846	0.242	0.182	2.88	95.55	90.20	26.43b
	W-Red	1.723b	37.82b	0.396	3.70	0.664	0.206	0.176	3.23	77.95	77.68	28.83b
	W-Far-red	1.738b	37.92b	0.351	3.86	0.859	0.257	0.180	3.05	94.63	92.55	23.45b
	Significance	***	**	NS	NS	NS	NS	NS	NS	NS	NS	**

Significant differences are indicated by letters at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ with *, ** and ***, respectively.
NS represents no significant differences.

Chapter 3 - Supplemental UV-A and UV-B Affect the Nutritional Quality of Lettuce and Tomato: Health-promoting Phytochemicals and Essential Nutrients.

Abstract

UV radiation plays an important role not only in plant growth and development but also in the accumulation of essential nutrients and health-promoting phytochemicals in plants. The main objective this study was to examine the effects of supplemental UV-A , UV-B, and UV-AB on the nutritional quality of lettuce (*Lactuca sativa*, cv. red leaf ‘New Red Fire’ and green leaf ‘Two Star) and tomato (*Solanum lycopersicum* L., cv. BHN-589) grown in a greenhouse. Supplemental UV radiation was provided by UV lamps during the 5-6 days prior to harvest. Supplemental UV-A radiation produced higher accumulation of total phenolic compounds and higher antioxidant capacity in red leaf lettuce compared to other treatments. Overall, supplemental UV-A had a stronger response than other UV treatments and control in the accumulation of many phenolic compounds including luteolin-7-glucoside, quecetin-3-glucoside, and apigenin-3-glucoside in the leaves of red leaf lettuce. However, UV-B and UV-AB had a negative response in the accumulation of many phenolic compounds including chlorogenic acid, luteolin-7-glucoside, quercetin-3-glucoside, and apigenin-3-glucoside in both red and green leaf lettuce varieties. In tomato fruits, supplemental UV-A had no effect on their total phenolic concentration. However, supplemental UV-B radiation for 3 h or UV-AB radiation up to 9 h exposure produced higher total phenolic concentration in the fruits compared to other supplemental UV treatments. Supplemental UV-AB (3 h exposure) was generally more effective than other UV treatments in increasing the accumulation of a number of phenolic compounds including chlorogenic acid, caffeic acid, chicoric acid, luteolin-7-glucoside, and other flavonoids in ripe tomato fruits. Supplemental UV-A produced higher accumulation of carotenoids including lutein and β-carotene than other supplemental UV treatments, while supplemental UV-AB increased the accumulation of lycopene in fully ripe tomatoes. With regard to the essential nutrients, green leaf lettuce was more responsive to the supplemental UV treatments than red leaf lettuce. All the supplemental UV treatments produced an increase in protein concentration in the leaves of green leaf lettuce. However, supplemental UV-AB produced a stronger response compared to the control and other UV treatments in increasing the accumulation of many nutrients including protein, phosphorus, potassium, sulfur, and zinc in green leaf lettuce 'Two Star'. However, supplemental UV-treatments did not affect the accumulation of any essential nutrients in fully ripe tomato fruits. [This statement contradicts the previous statement, in which you said supplemental UV-AB increased essential nutrients.] The results show that supplemental UV enhances the nutritional quality of lettuce in relation to both health-promoting phytochemicals and essential nutrients. Similarly, supplemental UV enhances nutritional quality in tomato fruits with higher accumulation of phenolic compounds and carotenoids than the control treatment.

Keywords: UV-A and UV-B lamps, lettuce, tomato, micronutrients, nutritional quality, phytochemicals, spectral quality, greenhouse

3.1 Introduction

UV radiation (UV-A and UV-B) plays an important role in the growth and development of plants. Of the total solar UV radiation reaching plants, the major part is UV-A radiation (315-400 nm), while the shorter wavelength UV-B (280-315 nm), which comprises only 5%, is more energetic and has been studied extensively with regard to its role in various plant functions including growth, morphogenesis, plant adaptation, and physiological processes such as photosynthesis and secondary metabolism (Teramura, 1983; Rozema et al., 1997; Tsormpatsidis et al., 2008). Impact of UV radiation on various aspects of vegetative and reproductive growth and the accumulation of secondary metabolites, including phenolic compounds and carotenoids in several crop species, has been reviewed by Huche-Thelier et al. (2016). On the one hand, UV-radiation, especially UV-B, can induce reactive oxygen species, which can be damaging to many organelles and macromolecules and impair photosynthetic function leading to reduced growth (Frohnmeyer and Staiger, 2003; Hollosy, 2002). On the other hand, it can also trigger the production of antioxidant species, which have a defensive function in plants (Braun and Tevini, 1993). UV radiation can induce a plethora of phenolic compounds, especially flavonoids which not only protect plants against high doses of UV-B and which also are antioxidants and have health-promoting qualities. They are known to reduce the risk of many common chronic and degenerative diseases including cardiovascular diseases, cancers, diabetes, and arthritis; more importantly they promote overall health in humans (Prior and Cao, 2000; Brit et al., 2001). Therefore, it is important to develop strategies to improve the nutritional quality of our food in relation to health-promoting phytochemicals and essential nutrients. The role of UV radiation in activating secondary metabolism has been reported in a number of plant species including lettuce (Tsormpatsidis et al., 2008). Crops grown in open fields with the benefit of solar radiation consisting of UV radiation have been shown to have increased health-promoting phytochemicals (Zhao et al., 2007; Oh et al, 2011). Thus, UV radiation can have an impact on health-promoting phytochemicals. But it is not clear whether they also have any effect on the accumulation of essential nutrients in plants, which can have major impacts on the overall nutritional quality of our food (Prior and Cao, 2000).

Malnutrition is a major global public health challenge arising from inadequate consumption of both macronutrients (calorie rich food; proteins) and many micronutrients (Müller and Krawinkel, 2005). Although it is a more serious problem in developing countries, it is prevalent in most regions of the world including the U.S. (LPI, 2018). The nutrients of major concern are protein (globally a billion people have chronic inadequate protein consumption) (Wu et al., 2014) and many micronutrients including calcium, iron, zinc, and many others (Ritchie and Roser, 2018; Tulchinsky, 2010). Efforts to mitigate the challenges of malnutrition have included primarily the consumption of nutrient-rich food and using supplements (Mülle and Krawinkel, 2005; Ritchie and Roser, 2018). Improving nutritional quality by developing nutrient-dense food is a critical challenge in addressing the issue of nutrient deficiency and malnutrition. Therefore, in this study, we investigated the effects of supplemental UV-A, UV-B, and UV-AB on the growth and nutritional quality of common vegetables, namely lettuce and tomato, in relation to their ability to increase health-promoting phytochemicals and essential nutrients.

3.2 Materials and Methods

3.2.1 Plant growing conditions

Seeds of two varieties of lettuce (*Lactuca sativa*), a red leaf, ‘New Red Fire’ and a green leaf, ‘Two Star’ were sown in 72-cell plug trays containing Metromix 360 soil mix, (Sungro Horticulture, Agawam, MA) in a growth chamber set at 22^{o}C (day/night) with a PAR photon flux of 274 μmol/m^2/s, a 12 h photoperiod and 60% relative humidity (May 5th, 2017 in spring season). One week old seedlings were then transplanted into pots (12 cm X 12 cm X 12 cm) containing Metro 360 soil mix and after 3 weeks, seedlings were then transferred to a greenhouse maintained at 25°C and 70% RH with an average sun light intensity of 719 μmol/m^2/s. Plants were irrigated 4 times a week and fertilized once a week with fertilized water (N:P:K-20-10-20) at 200 ppm of nitrogen. The total UV solar radiation in the greenhouse was measured using a Research Radiometer (ILT 5000, International Light Technologies, Inc., Peabody, MA) during mid-day at the canopy level. Plants were treated with supplemental UV just after sunset prior to harvest using UV lamps (UV-A 340 and UV-B 313EL, Q-Lab Corp., Cleveland, OH). Plants were treated with UV-A (8.11 W/m^2), and UV-B (1.97 W/m^2), and UV-AB (5.08 + 1.55 W/m^2) for 5 or 6 days prior to harvest as indicated in Table 3.1. UV exposure treatments were started on June 2nd, 2017. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications.

Tomato seeds (*Solanum lycopersicum* L. (cultivar ʹBHN-589ʹ)) were germinated in 32 cells plug trays containing metro-mix soil media (July 7th, 2017 in summer). The plants were grown in a growth chamber set at, 25 ± 2℃ temperature and 60% relative humidity and with a 16 h photoperiod and a PPFD (photosynthetic photon flux density) of 288 μmol/m^2/s for 4 weeks. The plants were then transferred to big pots (21.5 diameter x 21 height) with the same soil mix as above (Aug 8th, 2017 in summer). Transplanted seedlings were moved to a greenhouse, which was maintained at 25℃ and 70% relative humidity and were grown for 60 days before the supplemental UV treatments (October 4th, 2017). The plants were irrigated to pot capacity 3 times a week and fertilized once a week with fertilized water as described above. The average solar light intensity and the supplemental UV treatments were same as outlined for lettuce plants. UV-B and UV-AB treatments each consisted of 3 exposure periods, namely 3h, 6h and 9h with 1.5 h exposure/day, 6 days before harvest (Table 3.1). UV exposure treatments were started on October 5th, 2017. The experiment was conducted using a RCBD layout with 3 replications. Fruits were harvested after the UV treatments and were sorted into breaker and ripe stages, which were separately analyzed for various characteristics.

3.2.2 Growth characteristics

All the growth characteristics were measured at the time of harvest. The fresh weights of shoots and roots of lettuce were measured, and their dry weights were obtained after freeze-drying (Harvest Right, North Salt Lake, Utah) until a constant sample weight was reached. Leaf area was measured using a LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska). Specific leaf weight was calculated by dividing dry leaf weight by leaf area. Tomato fruits were harvested beginning in early October in 2017. Samples of lettuce leaves and tomato fruits were freeze-dried and stored at -20°C for further analyses.

3.2.3 Chlorophyll and carotenoids

The chlorophyll and carotenoid concentrations in lettuce leaves were determined according to the methods by Pora et al., (1989) and Chen et al., (2019) with some modifications. Lettuce leaf (0.03 g) samples were extracted with 3 mL 80% acetone for 25 min in an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT). The absorbance (A) of the extract was read at 663 nm, 645 nm, and 470 nm in a microplate reader (Synergy H1, BioTek, Winooski, VT). The leaf concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids were estimated using the following relationships:

$$\text{Chl a} = 12.72\, A_{663} - 2.59\, A_{645}$$

$$\text{Chl b} = 22.88\, A_{645} - 4.567\, A_{663}$$

$$\text{Total Chl a + b} = 20.3\, A_{645} + 7.22\, A_{663}$$

Carotenoids = (1000 A470 - 3.27Chl a - 104Chl b)/229

3.2.4 Anthocyanins

Lettuce leaf anthocyanins were extracted as outlined by Nakata et al. (2013) with some modifications. Lettuce leaf samples (0.02 g) were extracted twice with 0.5 mL of extraction buffer [methanol:acetic acid-45:5 v/v] using an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT). The supernatants were centrifuged at 12,000 x g for 5 min and the absorbance of supernatants was measured at 530 and 657 nm in a microplate reader (Synergy H1, BioTek, Winooski, VT, USA). Concentrations of anthocyanin in leaves were calculated using the following relationship: (Abs530/g D.W.) = [Abs530-(0.25 x Abs657)]x25. The anthocyanin concentration was expressed on a dry weight basis.

3.2.5 Total phenolic concentration and antioxidants capacity

The concentration of total phenolic compounds was determined using the modified Folin-Ciocalteu reagent method (Ainsworth and Gilespie, 2007). Ground freeze-dried lettuce leaf (0.04 g) and tomato fruit samples (0.4 g) were extracted in 4 mL of 80% acetone using an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT) for 20 min. A 1.5 mL aliquot of the extract was transferred to a centrifuge tube and kept in the darkness overnight at 4^{o}C. The extract was then centrifuged at 1000 rpm for 2 min and a 50 µL of the supernatant was mixed with 135 µL of distilled water, 750 µL diluted (1:10) Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), and 600 µL of 7.5% (w/v) Na_2CO_3. The mixture was vortexed and incubated in water bath at 45^{o}C for 15 min and was then allowed to cool at room temperature. The absorbance of the mixture was read at 765 nm (U-1100 Spectrophotometer, Hitachi Ltd. TokyoJapan). Gallic acid standards were prepared (Acros Organics, Fair Lawn, NJ) in 80% acetone with 3 replicates for each concentration. The amount of total phenolic concentration was quantified as GAE equivalent on the dry weight basis.

ABTS (aminobenzotriazole) decolorization assay was used to determine the total antioxidant capacity as outlined by Miller and Rice-Evans (1996) and Pennycooke et al. (2005). A 2.5 mM ABTS stock solution was used to generate ABTS * radical cations by adding 0.4 g of MnO_2 as an oxidizing agent and stirring continuously at room temperature. Excess MnO_2 was removed by filtering under vacuum first, and then using 0.22 μm syringe end filter. Then, the ABTS* solution was diluted to an absorbance value of 0.7 (±0.05) at 730 nm by using 5 mM PBS (phosphate buffer saline) at pH 7.4 and stored in water bath at 30^oC. One mL of ABTS* reagent was added to Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxyl acid) standards or samples and vortexed for 10 s and followed by 1 min of reaction time. The absorbance of the reaction mixture was measured at 730 nm. A PBS solution was used as a blank for each assay. The antioxidant capacity of samples was estimated as the Trolox equivalent.

3.2.6 Individual phenolic compounds

Freeze-dried and ground lettuce leaf and tomato samples (0.1 g and 0.4 g, respectively) were extracted overnight with 10 mL of 70% aqueous methanol on an orbital shaker (Benchmark, Edison, NJ) in the dark. The extract was centrifuged to collect the supernatant and the residue was further washed twice with methanol and centrifuged to get the final supernatant volume of 30 mL. A 2 mL aliquot of the supernatant was evaporated to dryness under streaming nitrogen gas and the residue was re-dissolved in 1 mL of 70% methanol and then filtered through 0.22 mm syringe end filter (MilliporeSigma, Burlington, MA). The phenolic compounds were quantified using a Shimadzu HPLC system (Kyoto, Japan) consisting of a DGU-20A3 degasser, a LC-20AB liquid delivery pump, a SIL-20ACHT auto-sampler, a CTO-20AC column oven, and a UV/Vis detector

(180-800nm). A C18 reversed phase column (250 mm L x 4.6 mm D, Waters, Milford, MA) was used to separate the phenolic compounds. The elution was conducted with a mobile phase A consisting of 5% formic acid in deionized water and mobile phase B consisting of 5% formic acids in 95% methanol at a rate of 0.8 mL/min and 31°C oven temperature. The gradient used for solvent B was 0-10% for 5 min, 10-40% for 25 min, 40-70% for 10 min, and 70% for 16 min before returning to 0%. The data were analyzed using the Shimadzu LC Solution Software (Kyoto, Japan). Concentrations of phenolic acids (gallic acid, chlorogenic acid, caffeic acid, and chicoric acid) and flavonoids [luteolin-7-glu (glucoside), quercetin-2-glu, apigenin-3-glu, and kampferol-3-glu] were expressed as equivalents of vanillic acid and quercetin, respectively.

3.2.7 Individual carotenoids

Accumulation of individual carotenoids was measured in tomato fruits from plants subjected to supplemental UV treatments. Samples from freeze-dried and ground fruit pericarp at breaker and fully ripe stages (0.3 g) were extracted with 6 mL of extraction solution (ethanol: hexane, 4:3, v/v) on an orbital shaker for 4 h. The extract was centrifuged in an Eppendorf centrifuge at 3950 rpm at 20℃ for 30 min. The supernatant was collected, and the residue was re-extracted with 8 mL hexane twice. The collected supernatant was washed with 30 mL distilled water first, and then 30 mL of 10% sodium chloride (NaCl) solution several times. The lipid layer was collected and 2 mL of this was evaporated in an Eppendorf vacufuge (Concentrator 5301, Hamburg, Germany) to dryness and was re-dissolved in 2 mL of methanol:MTBE (methyl tert-butyl ether, 1:1, v/v). The resulting solution was filtered through a 0.22 μm membrane filter before the HPLC analyses. All the extraction procedures were conducted under dim light in a transfer hood.

Carotenoids were separated and quantified using Shimadzu HPLC system (Kyoto, Japan) as described above using a YMC C30 reversed phase column. The mobile phase consisted of methanol:MTBE (7:3, v/v, Solvent A) and MTBE (100% Solvent B). The gradient of elution for solvent B was as follows: 0% to 10% in 6 min, 10% to 20% in 16 min, 20% to 70% in 26 min, 70% to 10% in 36 min. The carotenoids (lutein, β-carotene, and lycopene) were quantified at 450 nm using Shimadzu LC Solution Software (Kyoto, Japan).

3.2.8 Essential nutrients

Lyophilized and powdered lettuce leaf and tomato samples (0.15 g) were used to measure concentrations of essential nutrients. Concentrations of total carbon and nitrogen were determined using a LECO TruSpec CN combustion analyzer. Protein concentrations were derived from the leaf. Nitrogen concentrations using a conversion factor of 6.25 (Milton and Dintzis 1981). The concentrations of nutrients, including phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese, and zinc, were determined using an inductively coupled plasma (ICP) spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian, Australia PTY Ltd., Australia).

3.2.9 Statistical analysis

Data on treatment effects were analyzed using one-way Analysis of Variance (ANOVA) (SAS 9.4, Cary, NC and XLSTAT, Addinsoft, New York, NY) and the treatment means were compared using the Duncan's multiple range test at $p < 0.05$, < 0.01, and < 0.001.

3.3 Results and Discussion

3.3.1 Lettuce

3.3.1.a. Growth

The UV treatments were imposed on the red leaf ‘New Red Fire’ and green leaf ‘Two Star’ varieties of lettuce during the last 5 days of their growth period just prior to harvest (Table 3.1). Shoot and root biomasses of lettuce plants were determined after harvest. UV treatments did not affect the fresh or dry shoot biomass in both lettuce varieties. However, root biomass was affected by the UV treatment and was variety dependent.

In ‘New Red Fire’, both fresh and dry weights of root biomass were reduced by all the UV treatments, while, on the contrary, the fresh weights of roots in ‘Two Star’ increased due to UV-B treatment but with no effect on their dry weights by any UV treatments (Table 3.2). Although shoot mass was not affected by UV treatments in both varieties, plants in ‘New Red Fire’ were smaller under UV-B and UV-AB treatments because of their reduced leaf area. The total leaf area of these plants was about 86% or less relative to the control plants (Fig. 3.1). Krizek et al. (1998) examined the impact of solar UV radiation on red-leaf lettuce (cv. New Red Leaf) and found that both UV-A and UV-B reduced both fresh and dry weights of shoots. In our study, UV-B seems to have a negative impact on the growth of lettuce plants. Similar observations have also been made by others who found that high UV-B intensity reduced growth, including plant height, fresh and dry biomass, leaf area, and the relative growth rate, in maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) (Mark and Tevini, 1997; Singh et al., 2014). Caldwell et al. 1994 argued that a plant’s sensitivity to UV-B may depend on the spectral balance between visible and UV-A radiation. They found that shoot growth was reduced by UV-B only when visible and UV-A radiation levels were low in soybean (*Glycine max* Merr.) plants. When only visible light was low, UV-A appeared to mitigate the adverse effects of UV-B, but not with high visible light level. In our study, although supplemental UV-A had no impact on the leaf area in red leaf lettuce, supplemental UV-B and UV-AB reduced the total leaf area (Fig. 3.1). However, no significant impact of supplemental UV on the leaf area was observed in green leaf lettuce. The specific leaf weight (i.e., leaf dry mass/ unit area) in red leaf lettuce was higher in response to supplemental UV-B, although it resulted in reduced total leaf area compared to the control plants indicating that UV-B treated plants produced denser leaves. However, in green leaf lettuce, both supplemental UV-A and UV-AB treatments reduced the leaf specific weight, although there was no significant impact on the total leaf area by these treatments.

3.3.1.b. Chlorophyll, carotenoids, and anthocyanins

Leaf chlorophyll a concentration did not change in ‘New Red Fire’ lettuce with UV treatments while chlorophyll b level increased slightly with UV-AB treatment (Table 3.3). However, in ‘Two Star,’ UV treatments did not have any effect on the concentrations of both chlorophyll a and chlorophyll b. (Table 3.3). Similarly, leaf carotenoid levels were unaltered due to UV treatments in both lettuce varieties. With regard to leaf anthocyanin levels, UV treatments had no effect in ‘New Red Fire’ while there was an increase (44.2%) in ‘Two Star’ due to UV-B treatment compared to the control plants (Fig. 3.2). Similar observations were noted in lettuce in response to supplemental UV by Li and Kubota (2009). Other studies have also found similar response to UV-B in red leaf lettuce (Krizek et. al., 1998; Tsrompatsidis et al., 2008). However, control plants of red leaf variety ‘New Red Fire’ had higher anthocyanin concentration relative to their counterparts in the green leaf variety ‘Two Star.’ Exposure of lettuce plants to either UV-B or UV-AB tended to produce leaf discoloration (Fig. 3.2). This may be due to longer exposure of plants to these treatments. The negative impact of UV-B is expected, because of its relative high energy compared UV-A. Similar negative impacts of UV-B on leaf pigments have been reported in other plant species (Yao et al., 2006).

3.3.1.c. Phytochemicals

Both the total phenolic concentration and antioxidant capacity in ‘New Red Fire’ increased in response to supplemental UV-A while they were unaltered in response to both supplemental UV-B and UV-AB in New Red Fire (Fig. 3.3). Similar results have been reported in lettuce where both UV-A and UV-B induced higher accumulation of total phenolic compounds and the antioxidant capacity in lettuce and dropwort (Lee et al., 2013; Jeon et al., 2018). In our study, only supplemental UV-A was found to have a positive impact on the concentration of total phenolic compounds in ‘New Red Fire’ lettuce but not in ‘Two Star’ lettuce. The total phenolic concentration and antioxidant capacity in leaves of ‘New Red Fire’ increased by more than 56% and 86%, respectively, due to supplemental UV-A treatment compared to the control plants. However, no significant changes in either total phenolic concentration or antioxidant capacity were noted in green leaf ‘Two Star’ due to supplemental UV treatments.

Of the phytochemicals examined, chicoric acid was the dominant phenolic compound in both lettuce varieties irrespective of the supplemental UV treatments, (Fig. 3.4). Lettuce is rich in chicoric acid, which is an important health-promoting phytochemical known to play a significant role in fighting immune suppressing viral infections including HIV-1 (LaFemina et al., 1992). Supplemental UV-A treatment produced sharp increases in the concentration of luteolin-7-glu (121%), quercetin-3- glu (68%), and apigenin-3-glu (54%) in the leaves of red leaf ‘New Red Fire’ over the control. However, on the contrary, exposure of these plants to UV-B and UV-AB lowered the accumulations of luteolin-7-glu and apigenin-3-glu. Also, in ‘Two Star’, UV-A treatment produced higher accumulations of luteolin-7-glu. However, UV-AB treatment decreased the concentration of chlorogenic acid compared to the control. Overall, in ‘New Red Fire’, supplemental UV-A had a more positive impact in enhancing the accumulation of many flavonoids while supplemental UV-B and UV-AB had a negative impact on the accumulation of these flavonoids. Although the response is somewhat similar in ‘Two Star’ lettuce, the exception was that supplemental UV-B and UV-AB actually increased the accumulation of caffeic acid. Although the response of phenolic compounds to supplemental UV-A and UV-B radiation in lettuce were variable, the results from our study show that UV radiation plays a role in improving the accumulation of many phenolic compounds and thus enhancing the health-promoting qualities of lettuce. Krizek et al. (1998) found that in red leaf lettuce both UV-A and UV-B enhanced the concentration of UV absorbing compounds in plants, presumably flavonoids and other phenolic compounds which are known to have protective function against UV radiation in plants (Schmitz-Hoerner and Weissenbock, 2003). Similarly, in red leaf lettuce the photosynthetic function was higher in the presence of UV radiation than in its absence suggesting that higher accumulation of phenolic compounds under UV may have a protective function on the photosynthetic apparatus (Tsormpatsidis, 2008). Krizek et al. (1998) concluded that UV-B is more active in the induction of flavonoids than UV-A in red leaf lettuce. This is further supported by a study by Morales et al. (2010) where UV-B exclusion showed a decreased accumulation of many flavonoids in silver birch (*B. pendula* Roth) with lower expression of PAL (phenylalanine ammonia-lyase) gene in the leaves, which codes for the key gateway enzyme in the synthesis of phenolic compounds.

3.3.1.d. Essential nutrients

Red leaf ‘New Red Fire’ lettuce (control) was nutrient dense compared to green leaf ‘Two Star’ lettuce. It had higher concentrations of all the essential nutrients examined in this study (Table. 3.4). For example, the level of proteins in the leaves of ‘New Red Fire’ lettuce was more than 34% higher relative that of ‘Two Star’ lettuce. In addition, levels of many mineral nutrients, such as potassium, magnesium, sulfur, copper, manganese, and zinc, in red leaf lettuce were higher relative to those in green leaf lettuce. In particular, accumulation of copper, zinc, and manganese were notably higher in red leaf ‘New Red Fire’ lettuce relative the green leaf lettuce. The amount of copper, zinc, and manganese in red leaf lettuce was approximately 48%, 41% and 31% higher, respectively, compared to that in green leaf lettuce.

We examined the impact of UV treatments on the accumulation of essential nutrients in the leaves of red and green leaf lettuce varieties. In response to supplemental UV, leaf calcium and magnesium levels increased in ‘New Red Fire’ due to UV-A treatment while UV-B treatment resulted in only higher magnesium levels compared to the control plants (Fig. 3.5). However, overall, the UV response with regard to the accumulation of nutrients was much stronger in ‘Two Star’ than in ‘New Red Fire.’ Accumulation of most nutrients in ‘Two Star’ was higher with UV treatment relative to the control plants with exception carbon and iron. The accumulation of iron did not change with any UV treatment while carbon accumulation decreased in response to supplemental UV-B and UV-AB (Table 3.5). This suggests that photosynthetic activity can be adversely affected, especially by UV-B which is often reflected in reduced overall poor plant growth (Frohnmyer and Staiger, 2003).

Exposure of ‘Two Star’ to all the UV treatments resulted in higher accumulation of protein and sulfur in the leaves compared to the control (Fig. 3.6 and Table 3.5). The increase in protein (nitrogen) accumulation in the leaves ranged from 17 to 31%, with UV-AB producing the largest increase relative to the control plants. Specifically, supplemental UV-A treatment increased the accumulation of protein and sulfur by 17% and 21%, respectively, in the leaves of ‘Two Star’. Furthermore, supplemental UV-B treatment increased the accumulation of protein, phosphorus, potassium, calcium, magnesium, sulfur, manganese, and zinc, while supplemental UV-AB had a much stronger response in enhancing the accumulation of many nutrients including protein, phosphorus, potassium, sulfur, copper and zinc. UV-AB enhanced the concentration of phosphorus by 44%, potassium by 37%, copper by 31%, and zinc by 55% in leaves of ‘Two Star’ lettuce over the control plants. Although all the UV treatments have a positive role in enhancing many essential nutrients, UV-AB particularly is a potentially good agent in improving the nutritional quality of green leaf lettuce with regard to the essential nutrients. This indicates that both UV-A and UV-B, which are components of solar radiation, are essential for improving the nutritional quality of food we consume. From the perspective of mitigating the major nutrient deficiencies in the human diet, many of these nutrients, especially protein, play an important role. For example, treating green leaf ‘Two Star’ lettuce with supplemental UV-AB can increase the accumulation of protein by 31%. Protein is an important nutrient in human diet and its deficiency is widely prevalent in numerous countries in the world, especially in developing countries (Gerber et al., 2013). It is widespread in Asia and Africa. Approximately 43% of children (230 million) are afflicted by protein-energy deficiency, which can lead to serious health issues including loss of muscle mass, higher risk for bone fracture, edema, fatty liver disease, and many more diseases (de Onis et al., 1993).

Thus, overall, the results showed that supplemental UV can play a role in improving nutritional quality with regard to not only health-promoting phenolic compounds but also essential nutrients in lettuce.

3.3.2 Tomato

3.3.2.a. Phytochemicals in fruits

Total phenolic concentration and the antioxidant capacity of fully ripe tomato fruits were measured to identify the effects of supplemental UV-A, UV-B, and UV-AB in plants grown in a greenhouse. Tomato plants were treated with supplemental UV-A for 5 days and with UV-B and UV-AB for 3h, 6h or 9h (1.5h/day) prior to harvest. Supplemental UV-A had no effect on the total phenolic concentration in fully ripe tomato fruits (Fig. 3.7). Similarly, supplemental UV-B treatment for 3h or 6h did not affect the total phenolic concentration. However, longer exposure to UV-B for 9h and UV-AB for 3h or 9h increased the concentration of total phenolic compounds. Thus, a brief exposure (3h) of plants before harvest using a combination UV-A and UV-B could enhance the total phenolic concentration of tomato fruits. Although there was an increase in the total phenolic concentrations in the fruits due to UV treatments, it was not reflected by an increase in their antioxidant capacity. This is consistent with observations in a study where blocking UV-B in a greenhouse experiment did not affect the antioxidant capacity of tomato fruits in different tomato genotypes (Giuntini et al., 2005).

Individual phenolic compounds and carotenoids were measured in tomato fruits at breaker and fully ripe stages to identify the impact of supplemental UV-A, UV-B, and UV-AB in plants grown in a greenhouse. In tomato fruits (pericarp), the dominant phenolic compound was chlorogenic acid of all the phenolic compounds monitored both at the breaker and fully ripe stages (Fig. 3.8). A higher accumulation of chlorogenic acid was found in fruits at breaker stage than at the fully ripe stage. At the breaker stage, supplemental UV had a negative impact on the accumulation of caffeic acid and luteolin-7-glu in fruits. In fact, on the one hand, supplemental UV treatment did not have any positive impact on the concentration of any phenolic compounds examined in fruits at the breaker stage. On the other hand, supplemental UV treatment induced higher accumulation of many phenolic compounds when fruits were fully ripe, which is notably a beneficial characteristic with regard to their health-promoting ability in our diet. In fully ripe fruits, supplemental UV treatment induced higher accumulations of a number of phenolic compounds. Treatment of plants with supplemental UV-A or UV-AB (3h) increased the chlorogenic acid concentration in fully ripe fruits by more than 50% over the control. Supplemental UV-A also increased the concentrations of chicoric acid and apigenin-3-glu in fully ripe fruits. Generally, supplemental UV-AB (3h) was more effective in increasing the accumulation of a number of phenolic compounds including chlorogenic acid, caffeic acid, chicoric acid, luteolin-7-glu, apigenin-3-glu, and kaempferol-3-glu. However, in the case kaempferol-3-glu, supplemental UV-B treatment for longer exposure up to 9h produced a higher accumulation compared to the control. Overall, higher accumulations of phenolic compounds occurred in fruits at the fully ripe stage when plants were subjected to supplemental UV-AB, especially for short period of time (3h).

The accumulation of key carotenoids in tomato fruits at the breaker and fully ripe stages in response to supplemental UV treatments is presented in Figure. 3.9. At the breaker stage, exposure of plants to supplemental UV-B and UV-AB for various lengths of time improved the concentration of β-carotene in fruits but with no impact on the concentration of lutein. There were no detectable levels of lycopene in fruits at the breaker stage. At the fully ripe stage, supplemental UV-A produced the highest concentration of lutein and β-carotene in the fruits. Also, lutein concentration in fruits was higher in response to supplemental UV-B (3h) and to UV-AB at all exposures up to 9h compared to the control. Similarly, accumulation of β-carotene was higher in response supplemental UV-AB (3h). Furthermore, an increase in lycopene occurred in ripe fruits in response to UV-AB at only 9h exposure. Similar positive results were observed in tomatoes grown in poly-tunnels where fruits contained higher lycopene and β-carotene concentration with higher UV transmittance (Živanović et al., 2017). The results from our study suggest that supplemental UV treatment, especially (UV-A and UV-AB), of tomato plants just prior to harvest has a beneficial impact on the key carotenoids such as lycopene, lutein, and β-carotene that play an important role in maintaining eye health, in reducing the risks of some cancers, and help maintain the overall health and wellbeing in humans (Giovannucci et al., 2002; Abdel-Aal et al., 2013

3.3.2.b. Essential nutrients in fruits

The essential nutrients, including nitrogen (protein), phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese, and zinc, were measured in tomato fruits at breaker and fully ripe stages in response to supplemental UV. Treatment of plants with supplemental UV-A, UV-B, and UV-AB did not produce any significant effect on the accumulation of any of the nutrients in fruits at either breaker or fully ripe stage examined in this study (Table 3.6).

In summary, the response of lettuce to supplemental UV-A was variable and dependent on the variety. Supplemental UV-A enhanced the accumulation of many phenolic compounds such as chlorogenic acid, luteolin-7-glucoside, quercetin-3-glucoside, and apigenin-3-glucoside in red leaf lettuce. However, UV-B and UV-AB increased the accumulation of many essential nutrients including protein, phosphorus, potassium, calcium, sulfur, and zinc in green leaf lettuce. Similarly, supplemental UV-AB played an important role in enhancing the accumulation of a number of phenolic compounds such as chlorogenic acid, caffeic acid, chicoric acid, luteolin-7-glucoside, apigenin-3-glucoside, and kaempferol-3-glucoside in fully ripe tomato fruits. In addition, supplemental UV treatment resulted in higher accumulation of carotenoids including lutein, β-carotene, and lycopene in fully ripe fruits. Thus, the results show that supplemental UV radiation can enhance the nutritional quality of lettuce and tomato by increasing the accumulation of health-promoting phenolic compounds and carotenoids.

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Figures and Tables

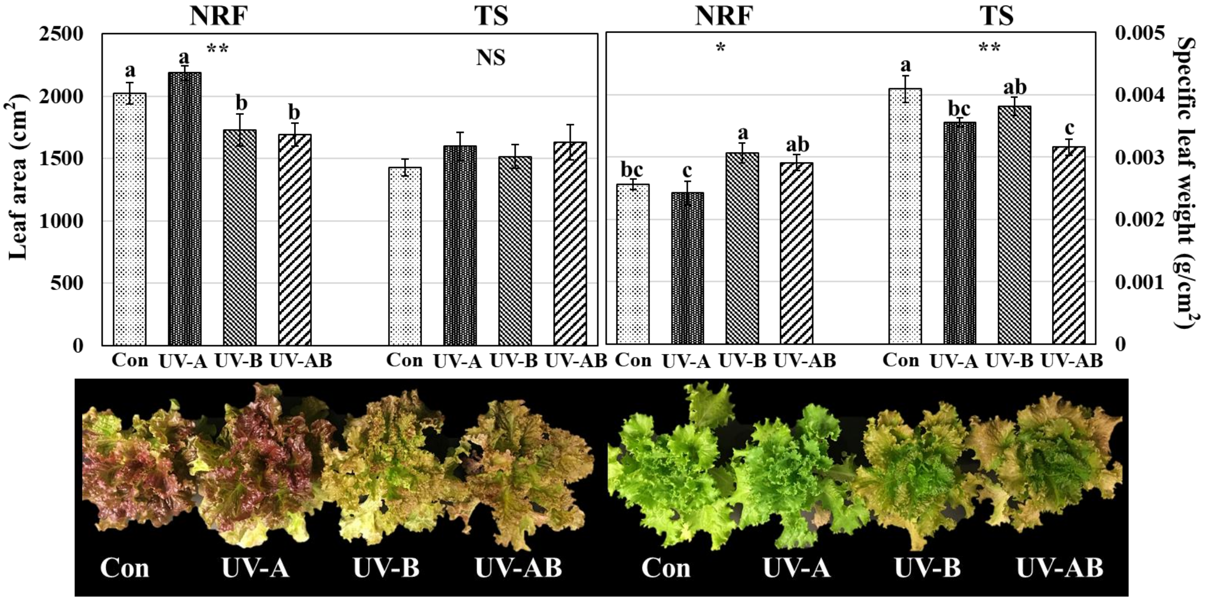


Figure 3. 1. Upper panel: The effects of the supplemental UV treatments on the leaf area and specific leaf weights for 'New Red Fire' (NRF) and 'Two Star' (TS) measured at the time of harvest. Control (con) plants did not receive any supplemental UV. Significant differences are indicated by * and ** at $p \leq 0.05$ and $p \leq 0.01$, respectively. NS indicates lack of significant differences. Bars (treatment means + SE) with the same letter (s) show no significant treatment differences within a variety. Bottom panel: Growth characteristics of control and UV treated red and green lettuce plants at the time of harvest.

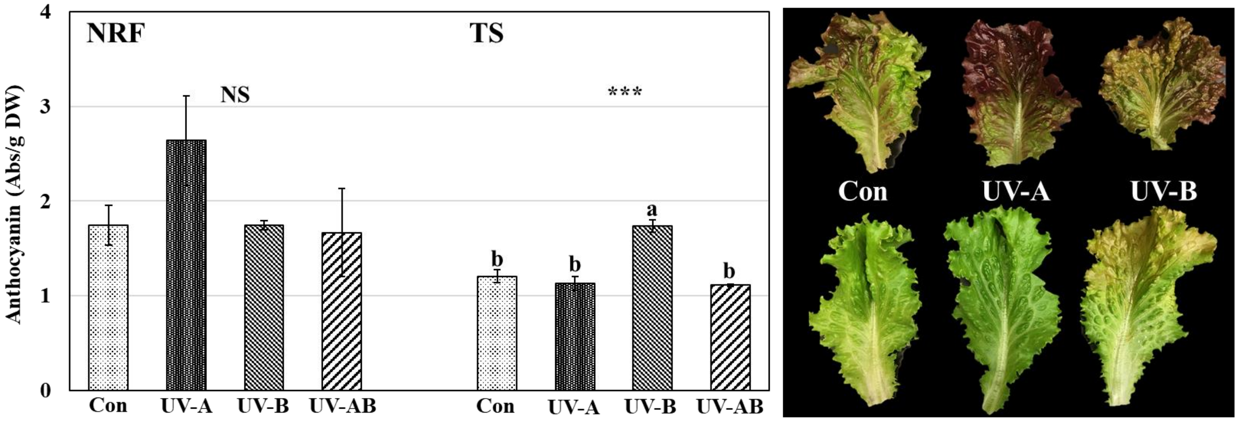


Figure 3. 2. Anthocyanin concentrations in the leaves at the time of harvest in ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) subjected to supplemental UV radiation. Photographs on the right show leaf color of red (top row) and green (bottom row) lettuce treated with supplemental radiation. Control (con) plants did not receive any supplemental UV treatment. Bars (treatment means + SE) with the same letter (s) show no significant treatment differences within a variety. Significant difference is indicated by * ** at $p < 0.001$. NS indicates lack of significant difference.

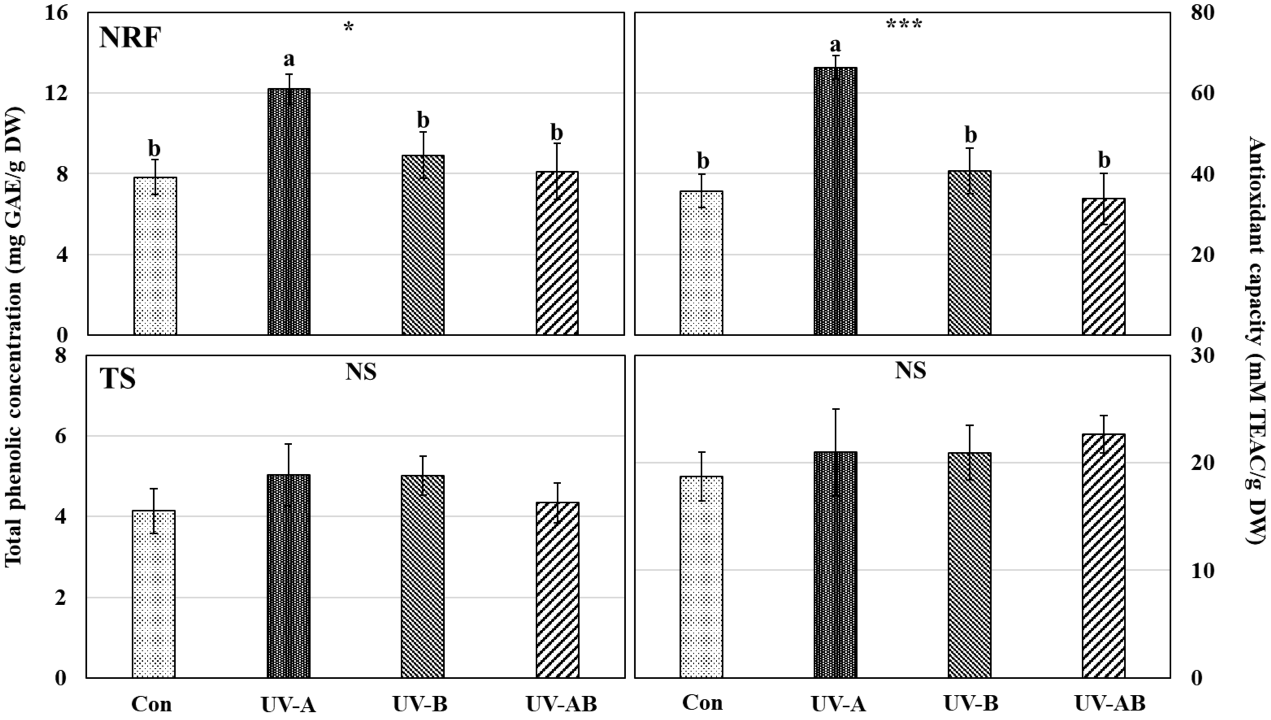


Figure 3. 3. Total phenolic concentration and antioxidant capacity at the time of harvest in 'New Red Fire' (NRF) and 'Two Star' (TS) subjected to supplemental UV radiation. Bars (treatment means + SE) with the same letter (s) show no significant treatment differences within a variety. Significant differences are indicated by * and * ** at $p < 0.5$ and $p < 0.001$, respectively. NS indicates lack of significant differences.

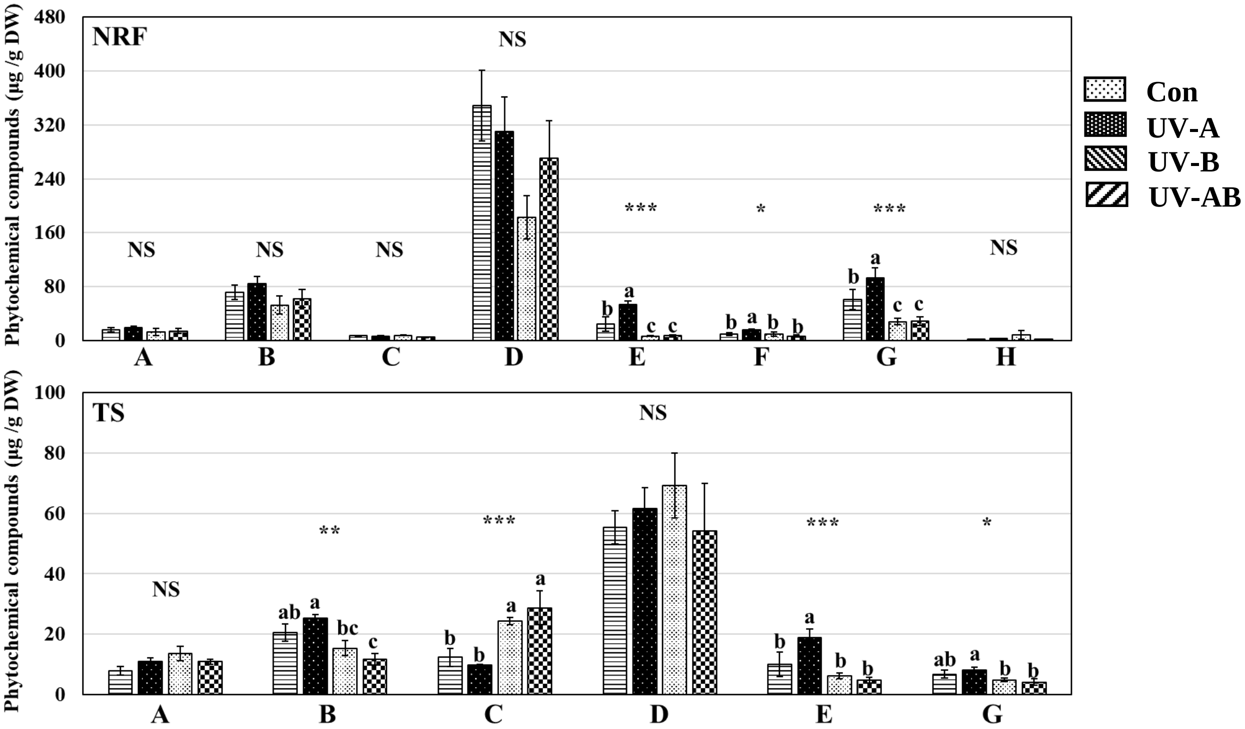


Figure 3. 4. Individual phenolic compounds at the time of harvest in 'New Red Fire' (NRF) and 'Two Star' (TS) subjected to supplemental UV radiation. Phenolic compounds included gallic acid (A), chlorogenic acid (B), caffeic acid (C), chicoric acid (D), luteolin-7-glucoside (glu) (E), quercetin-3-glu (F), apigenin-3-glu (G), kaempferol-3-glu (H). Bars (treatment means + SE) with the same letter (s) show no significant treatment differences. Significant differences are indicated by *, ** and *** at $p < 0.05$ $p < 0.01$, and $p < 0.001$, respectively. NS indicates lack of significant differences.

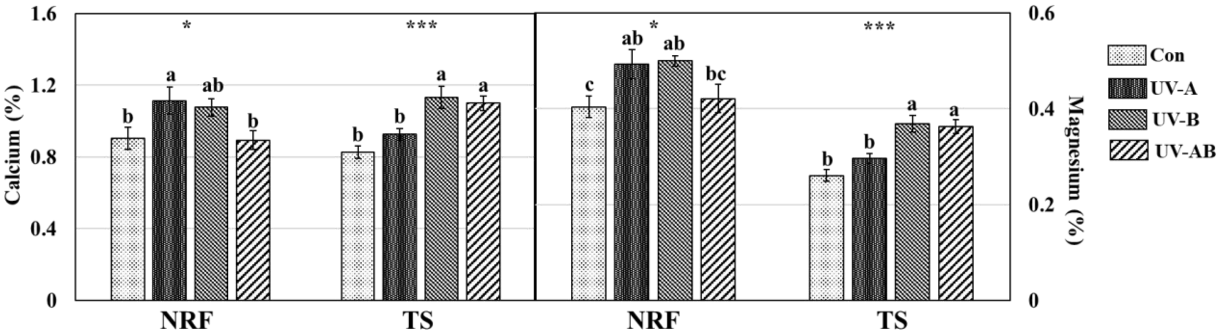


Figure 3. 5 Concentrations of calcium and magnesium in leaves at the time of harvest in 'New Red Leaf' (NRF) and 'Two Star' (TS) lettuce subjected to supplemental UV radiation. Bars (treatments + SE) with the same letter (s) show no significant treatment differences. Significant differences are indicated by * and *** at $p < 0.05$ and $p < 0.001$, respectively

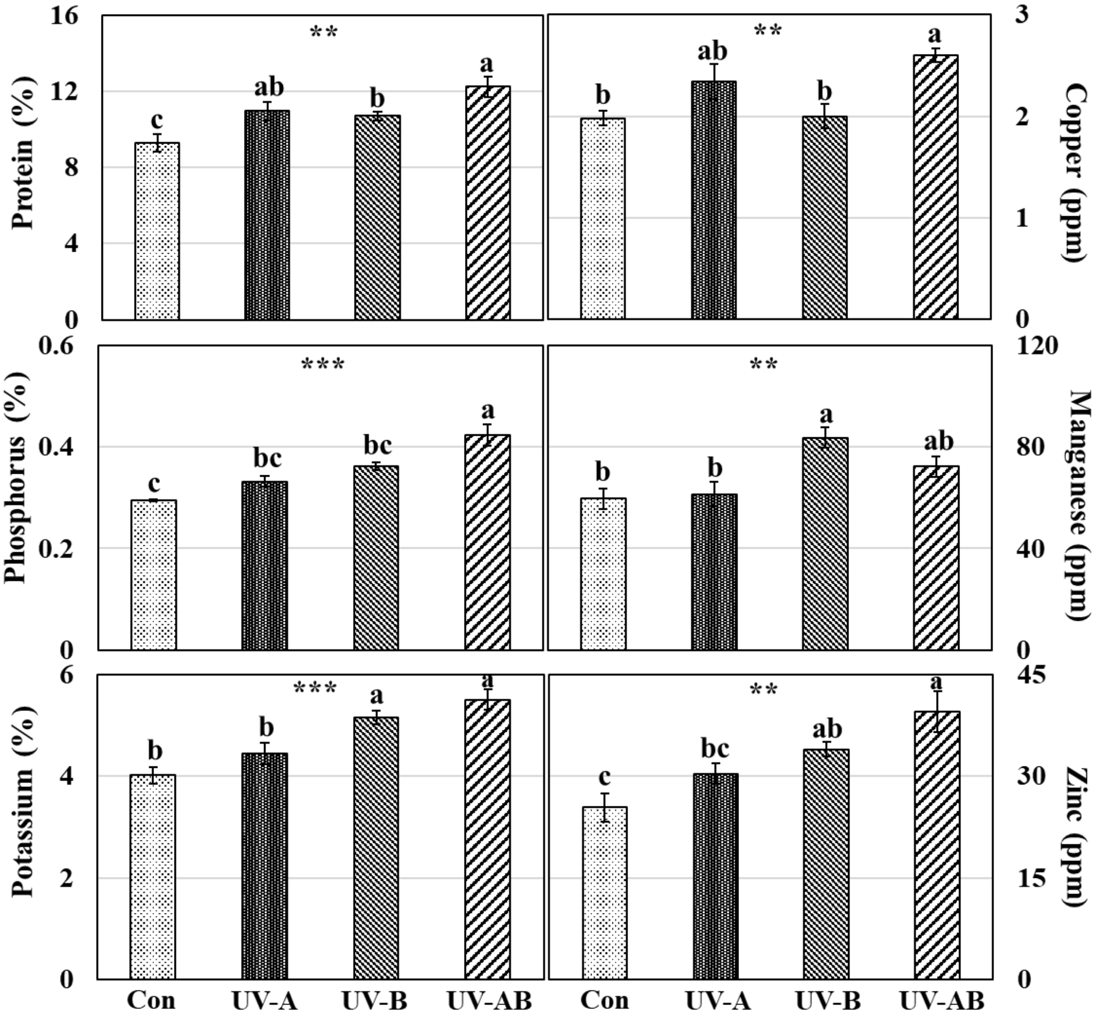


Figure 3. 6 Concentrations of essential nutrients in leaves of 'Two Star' (TS) lettuce subjected to supplemental UV radiation at the time of harvest. Protein concentration was derived from total nitrogen in the leaves. Bars (treatments + SE) with the same letter (s) show no significant treatment differences. Significant differences are indicated by ** and *** at $p < 0.01$ and $p < 0.001$, respectively**.**

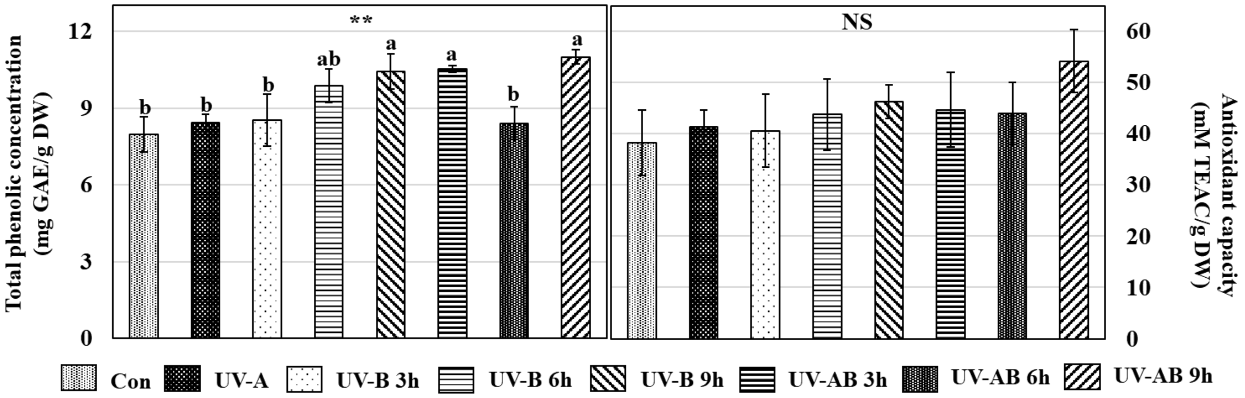


Figure 3. 7 Total phenolic concentration and antioxidant capacity of fully ripe tomato fruits. Plants were treated with supplemental UV-A and UV-B and UV-AB for 3h, 6h and 9h. Control plants (con) did not receive any UV treatment. Bars (SE) with the same letter (s) show no significant treatment differences. Significant difference is indicated by ** at $p < 0.01$. NS indicates lack of significant differences.

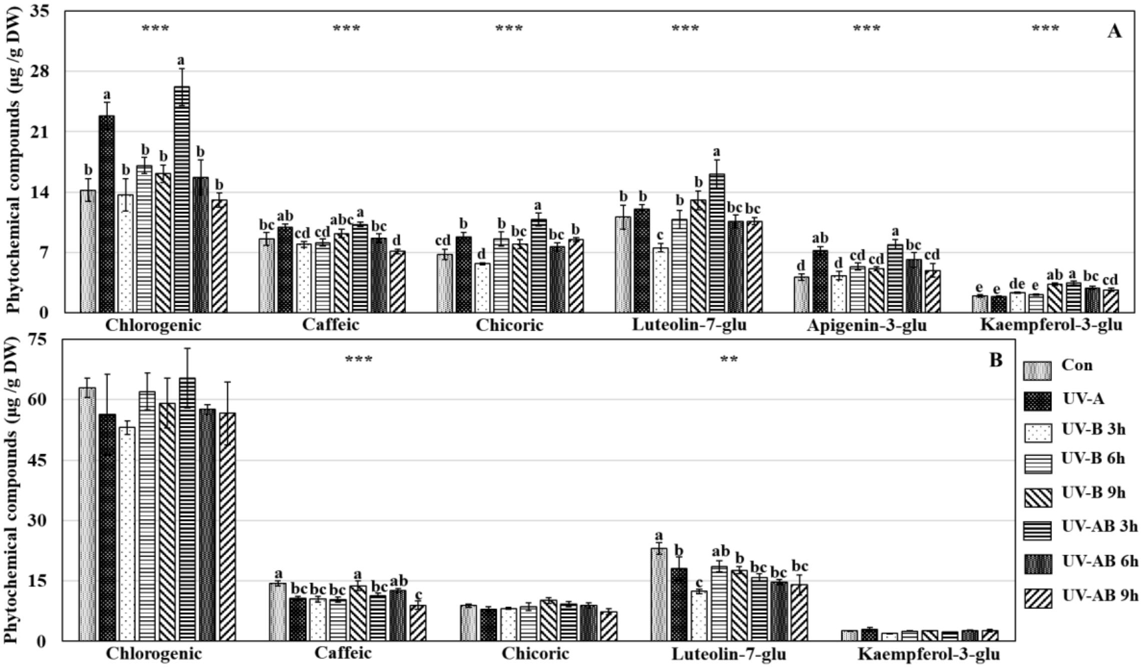


Figure 3. 8 Individual phenolic compounds in tomato fruits. Plants were treated with supplemental UV-A, and UV-B and UV-AB for 3h and 6h. Control plants (con) did not receive any UV treatment. Phenolic compounds included chlorogenic acid, caffeic acid, chicoric acid, luteolin-7-glu, apigenin-3-glu, kaempferol-3-glu in fully ripe (A) and breaker (B) fruits. Bars (SE) with the same letter (s) show no significant treatment differences. Significant differences are indicated by ** and *** at $p < 0.01$ and $p < 0.001$, respectively. NS indicates lack of significant differences.

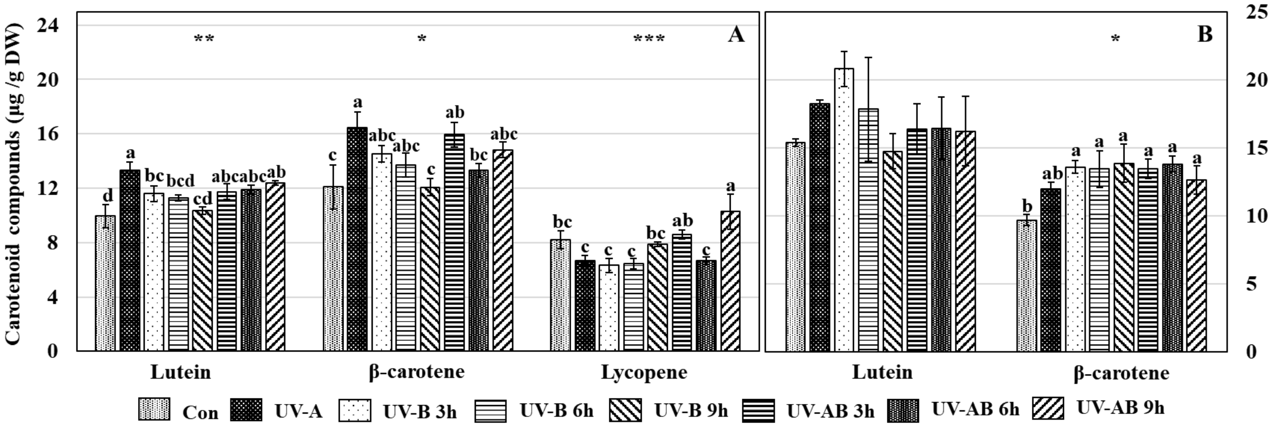


Figure 3. 9 Individual carotenoids compounds in tomato fruits. Plants were treated with supplemental UV-A, and UV-B and UV-AB for 3h and 6h. Control plants (con) did not receive any UV treatment. Carotenoid compounds included as lutein, β-carotene, and lycopene from fully ripe (A) and breaker (B) fruits. Bars (SE) with the same letter (s) show no significant treatment differences. Significant differences are indicated by ** and *** at $p < 0.01$ and $p <0.001$, respectively. NS indicates lack of significant difference.

Table 3. 1 Supplemental UV radiation treatment schedule for lettuce and tomato plants grown in greenhouse. The daily treatments were initiated after the sunset just prior to harvest. UV exposure started on June 2nd, 2017 for lettuce and on October 5th, 2017 for tomato.

	Supplemental radiation	Treatment schedule					
		Day 1	Day 2	Day 3	Day 4	Day 5	
Lettuce	Control	-	-	-	-	-	
	UV-A	24h	24h	24h	24h	24h	
	UV-B	-	2h	2h	2h	2h	
	UV-AB	-	2h	2h	4h	4h	
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Tomato	Control	-	-	-	-	-	-
	UV-A	24h	24h	24h	24h	24h	24h
	UV-B 3h	1.5h	1.5h				
	UV-B 6h	1.5h	1.5h	1.5h	1.5h		
	UV-B 9h	1.5h	1.5h	1.5h	1.5h	1.5h	1.5h
	UV-AB 3h	1.5h	1.5h				
	UV-AB 6h	1.5h	1.5h	1.5h	1.5h		
	UV-AB 9h	1.5h	1.5h	1.5h	1.5h	1.5h	1.5h

Table 3. 2 Shoot and root fresh and dry weights for lettuce varieties, ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) subjected to supplemental UV radiation for 5 days before harvest. Control plants did not receive UV radiation.

		Shoot		Root	
		FW (g/plant)	DW (g/plant)	FW (g/plant)	DW (g/plant)
New Red Fire	Control	71.14	5.20	16.46 a	1.18 a
	UV-A	82.38	5.32	9.82 b	0.75 b
	UV-B	62.93	5.33	10.85 b	0.71 b
	UV-AB	61.81	4.91	10.33 b	0.79 b
	Significance	NS	NS	**	*
Two Star	Control	67.42	5.83	11.18 b	0.76
	UV-A	72.64	5.68	13.57 ab	1.05
	UV-B	69.93	5.76	17.35 a	1.13
	UV-AB	73.93	11.96	11.96 b	1.02
	Significance	NS	NS	*	NS

Significant differences are indicated by * and ** at $p < 0.05$ and $p < 0.01$, respectively (n = 4)
NS indicates lack of significant differences. Means followed by same letter (s) in a column are not significantly different.

Table 3. 3 Concentrations of chlorophyll a and b, total chlorophyll, and carotenoids in the leaves of ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) subjected to supplemental UV radiation treatments. Measurement were made at the time of harvest.

	New Red Fire				Two Star			
	Chl a	Chl b	Total Chl	Carotenoid	Chl a	Chl b	Total Chl	Carotenoid
	(μg/g DW)				(μg/g DW)			
Control	161.2	4.77 bc	215.8	0.76	253.6	4.78	339.5	1.33
UV-A	202.6	4.59 c	267.9	0.98	288.4	4.80	386.8	1.38
UV-B	165.3	4.99 ab	224.4	0.86	267.2	5.01	363.4	1.37
UV-AB	113.8	5.18 a	157.1	0.59	278.4	4.96	377.4	1.46
Significance	NS	**	NS	NS	NS	NS	NS	NS

Significant differences are indicated by * and ** at $p < 0.05$ and $p < 0.01$, respectively (n = 4)
NS indicates lack of significant differences. Means followed by the same letter (s) in a column are not significantly different.

Table 3. 4 Concentrations of essential nutrients in leaves of ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) lettuces at the time of harvest. Protein concentration was derived from total the nitrogen concentration in the leaves**.**

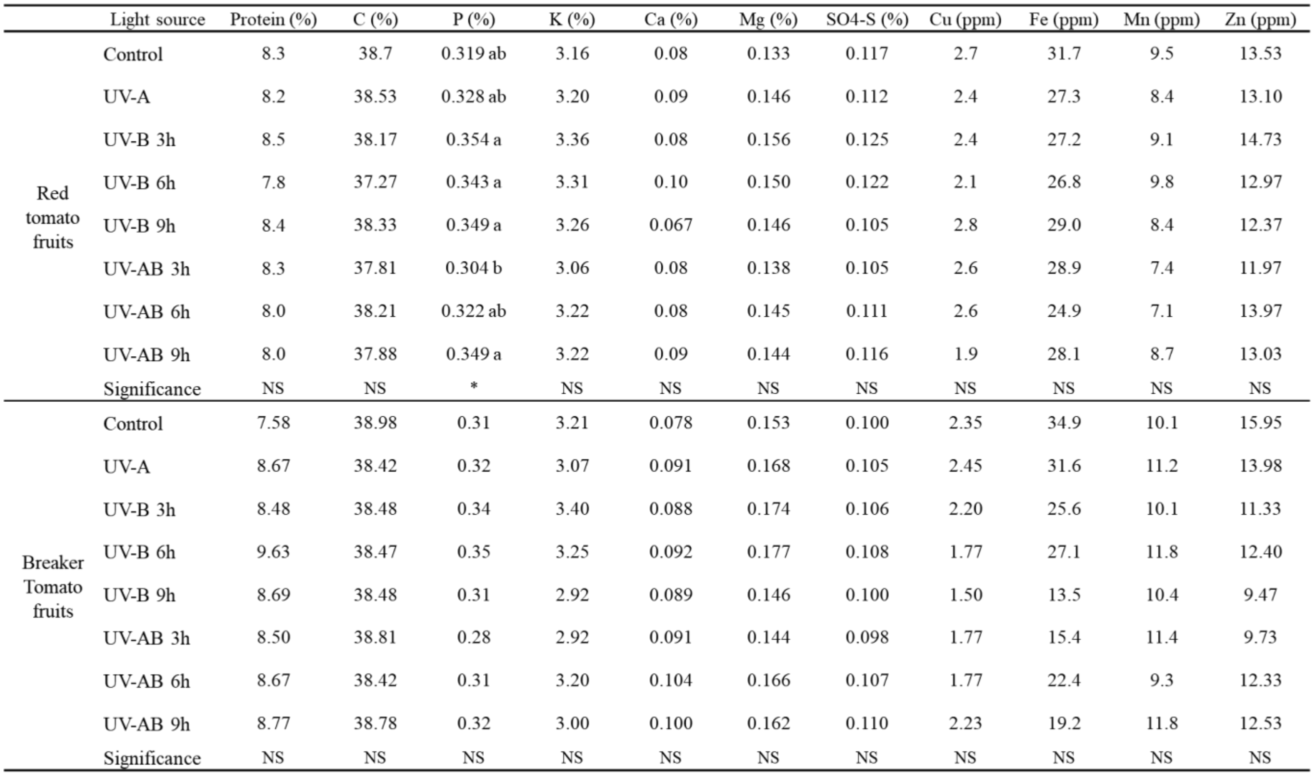
	Protein (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO_4-S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
New Red Fire	14.63	38.83	0.54	5.74	0.90	0.40	0.20	2.94	112.1	87.66	43.74
Two Star	10.88	38.69	0.29	4.01	0.83	0.26	0.14	1.98	96.4	59.7	25.4

Table 3. 5 Concentrations of carbon, sulfur, and iron in leaves of 'Two Star' (TS) at the time of harvest in subjected to supplemental UV radiation.

	Two Star		
	C (%)	SO4-S (%)	Fe (ppm)
Control	38.69 a	0.14 c	96.4
UV-A	39.00 a	0.17 b	111.1
UV-B	37.54 b	0.19 b	100.0
UV-AB	37.62 b	0.21 a	150.3
Significance	**	***	NS

Significant differences are indicated by ** and *** at $p < 0.01$ and $p < 0.001$, respectively (n = 4).
NS indicates lack of significant differences. Means followed by same letter (s) in a column are not significantly different.

Table 3. 6 Concentrations of essential nutrients in tomato fruits from plants subjected to supplemental UV radiation.



	Light source	Protein (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO4-S (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Red tomato fruits	Control	8.3	38.7	0.319 ab	3.16	0.08	0.133	0.117	2.7	31.7	9.5	13.53
	UV-A	8.2	38.53	0.328 ab	3.20	0.09	0.146	0.112	2.4	27.3	8.4	13.10
	UV-B 3h	8.5	38.17	0.354 a	3.36	0.08	0.156	0.125	2.4	27.2	9.1	14.73
	UV-B 6h	7.8	37.27	0.343 a	3.31	0.10	0.150	0.122	2.1	26.8	9.8	12.97
	UV-B 9h	8.4	38.33	0.349 a	3.26	0.067	0.146	0.105	2.8	29.0	8.4	12.37
	UV-AB 3h	8.3	37.81	0.304 b	3.06	0.08	0.138	0.105	2.6	28.9	7.4	11.97
	UV-AB 6h	8.0	38.21	0.322 ab	3.22	0.08	0.145	0.111	2.6	24.9	7.1	13.97
	UV-AB 9h	8.0	37.88	0.349 a	3.22	0.09	0.144	0.116	1.9	28.1	8.7	13.03
	Significance	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
Breaker Tomato fruits	Control	7.58	38.98	0.31	3.21	0.078	0.153	0.100	2.35	34.9	10.1	15.95
	UV-A	8.67	38.42	0.32	3.07	0.091	0.168	0.105	2.45	31.6	11.2	13.98
	UV-B 3h	8.48	38.48	0.34	3.40	0.088	0.174	0.106	2.20	25.6	10.1	11.33
	UV-B 6h	9.63	38.47	0.35	3.25	0.092	0.177	0.108	1.77	27.1	11.8	12.40
	UV-B 9h	8.69	38.48	0.31	2.92	0.089	0.146	0.100	1.50	13.5	10.4	9.47
	UV-AB 3h	8.50	38.81	0.28	2.92	0.091	0.144	0.098	1.77	15.4	11.4	9.73
	UV-AB 6h	8.67	38.42	0.31	3.20	0.104	0.166	0.107	1.77	22.4	9.3	12.33
	UV-AB 9h	8.77	38.78	0.32	3.00	0.100	0.162	0.110	2.23	19.2	11.8	12.53
	Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Significant differences are indicated by * at $p < 0.05$ (n = 4)

NS indicates lack of significant differences. Means followed by same letter (s) in a column are not significantly different.

Protein concentration was derived from total leaf nitrogen.

Chapter 4 - The Effect of Supplemental UV-A LEDs on the Nutritional Quality of Lettuce Grown in Growth Chambers

Abstract

The major part of solar UV radiation is UV-A, which affects many plant functions including secondary metabolism. Many secondary metabolites, which are health promoting phytochemicals, and essential nutrients are an important component of a healthy diet. UV-A response in plants is dependent on the level of photosynthetically active radiation (PAR) and, typically, PAR levels in growth chambers are relatively low unlike in open fields or even in greenhouses. We examined the effect of supplemental UV-A radiation via LEDs on the nutritional quality of lettuce (*Lactuca sativa* L., cv. red leaf 'New Red Fire' and green leaf 'Two Star') grown in growth chambers. Plants were subjected UV-A LEDs with filters and lens to alter the irradiance on the canopy. Supplemental UV-A did not affect many growth characteristics including shoot fresh weight, leaf area, and number of leaves/plants. It suppressed the accumulation of total phenolic compounds and the accumulation of many flavonoids including luteolin-7 glucoside, quercetin-3-glucoside, and apigenin-3-glucoside in red leaf lettuce. In contrast, supplemental UV-A increased the accumulation of many essential nutrients, including protein, phosphorus, potassium, and calcium, in both lettuce varieties. The results suggest that low PAR can influence the UV- A response. That is, if the PAR is low, little or no impact on growth and the accumulation of many phenolic compounds in lettuce occurs with UV-A addition. Therefore, high levels of PAR along with UV-A radiation are needed to produce positive plant responses in terms of better growth and higher accumulation of health-promoting phytochemicals.

Keywords: spectral quality, UV-A LEDs, phytochemicals, phenolic compounds, essential nutrients.

4.1 Introduction

UV-A, which makes up a major part of the solar UV radiation, plays an important role in modulating many aspects of plant growth and morphogenesis. It can induce a number of secondary metabolites, notably phenolic compounds including flavonoids (Krizek, 2004; Verdaguer et al., 2017). These phenolic compounds, especially flavonoids, absorb UV and prevent inhibitory actions of high doses of UV on plant functions. The damaging effects of UV-B and its interaction with UV-A on plant growth and metabolism have been well documented (Miercki and Teramura, 1984; Cen and Bornman, 1990). In addition to positive influences of phenolic compounds on plants, they also play in important role in promoting human health (Steinmetz and Potter, 1996; Prior and Cao, 2000: Rajashekar et al., 2009). Thus, spectral characteristics of visible and UV spectra can be used to improve the nutritional quality of our diet. Indeed, many studies have focused on manipulating the light characteristics, including UV, both in the field and in protective environments to improve the nutrition quality of food especially in relation to the phytochemical content (Luthria et al., 2006; Tsompatsidis et al., 2008). However, it is not clear if supplemental UV radiation using LEDs impacts the composition of essential nutrients in plants needed for human health.

Many horticultural crops are being grown under protective environmental conditions worldwide, which allows for easy manipulations of light characteristics (Tsompatsidis et al., 2008; Morales et al., 2010; Taulavuori et al. 2018). In addition, the rapid advancements in light emitting diodes (LEDs) has spurred the adoption of this technology as a lighting source in crop production in controlled environments (Kopsell et al., 2015). LEDs are solid state devices, and they are more energy efficient and long lasting than conventional lighting. More importantly, they allow for the precise control of spectral quality that is important in modulating the light characteristics for improved crop growth with high nutritional quality. UV-LEDs have been used to understand the role of UV in its role in regulating plant growth and behavior, and more importantly to improve the nutritional quality of horticultural food crops including lettuce (Bantis. et al., 2016; Li and Kubota, 2009; Taulavuori et a., 2017).

Although there are many studies examining the effects of UV-A and UV-B on plant functions, little is known about the interactions of UV with photosynthetically active radiation (PAR). PAR has been known to alleviate the inhibitory role of UV-B. Thus, higher PAR can reduce the negative impact of UV-B in plants (Krizek, 2004; Verdaguer et al., 2017). Interaction of PAR and UV radiation can modulate a plant’s response to light, and a lower PAR can promote negative effects of UV (Krizek et al., 2004). This observation is also supported by many field and greenhouse studies, where PAR levels are high and plants’ exposure to UV has been demonstrated to improve their phytochemical content (Zhao et al., 2007; Oh et al., 2011). Supplemental UV-A has a positive impact on the accumulation of many phenolic compounds, especially under field or greenhouse conditions, where PAR levels are fairly high. However, the response in growth chambers is different or even inhibitory, because growth chambers typically provide low PAR (Krizek, 2004; Brazaityte et al., 2015; Bantis et al., 2016). Thus, it is important to consider the interaction between PAR and UV-A to understand the role played by UV in modulating plant growth and other plant functions. Therefore in this study, we grew lettuce under low PAR in growth chambers with supplemental UV-A to examine the impact of UV-A on the nutritional quality to simulate the typical growth chamber conditions where PAR levels are low (Brazaityte et al., 2015; Bantis et al., 2016).

To date, most studies involving supplementing UV have primarily focused in improving plant growth and improving nutritional quality relating to phenolic compounds and other antioxidants. Little work has been done related to the essential nutrients. It is unclear if supplementing UV-A using LEDs under low PAR can improve the nutritional quality of lettuce in relation to essential nutrients. Therefore, our main objective of this study was to examine the effects of supplemental UV-A LEDs with scatter filters on the nutritional quality of lettuce grown under low PAR in growth chambers in relation to their ability to increase health-promoting phytochemicals (phenolic compounds) and essential nutrients.

4.2 Materials and Methods

4.2.1 Plant materials and growing conditions

Seeds of 2 lettuce varieties (*Lactuca sativa* L. cv. red leaf 'New Red Fire' and green leaf 'Two Star') were sown (March 2nd, 2018) in a 72 plug seedling trays containing a soil mix (Metromix 360, Sungro Horticulture, Agawam, MA) in a growth chamber set at 22 °C with a PPFD (photosynthetic photon flux density) of 244 µmol/m^2/sec (PAR) with 12 h photoperiod and 60% of relative humidity. Two-week old seedlings were transplanted (March 16th, 2018) to pots (12 cm X 12 cm X 12 cm) containing the above soil mix (1 seedling/pot). Plants were watered every 2 days with a fertilizer solution (N:P:K; 20:10:20) at 200 ppm of N once a week. Plants were rearranged every other day within the growth chamber so as to reduce the variability in irradiance on the plant canopy.

One week before harvest (April 6th, 2018), plants were subjected to UV-A treatment using custom built LEDs (375 nm) with special lens in a growth chamber (BDR16, Conviron Company, Winnipeg, Canada) with the background fluorescent lighting providing approximately a PPFD (photosynthetic photon flux density) of 190 µmol/m^2/s. LED bays (4 x 4) were built with lenses to modulate the distribution of UV-A radiation on the plant canopy. The treatments consisted of supplemental LEDs, supplemental LED with 5° lens (focus lens), and LED with scatter lens. PAR was measured at the canopy level using a Quantum Radiometer Photometer (LI-185B, LI-Cor, Inc., Lincoln, NE). UV-A radiation was measured using a Research Radiometer (ILT 5000, International Light Technologies, Inc., Peabody, MA) at 9 random sites within each treatment. Plants were randomly assigned under each treatment in a completely randomized design with 4 replications.

4.2.2 Plant growth

Plant growth, including fresh and dry weights of shoots, number of leaves, and leaf area, was measured after 3 days and 6 days of treatment. Dry weights were obtained by freeze-drying (Harvest Right, North Salt Lake, UT) the samples up to 16 h or until a constant dry weight was obtained. Leaf area was determined by a leaf area meter (LI-3100, Li-Cor, Lincoln, NE, USA). Freeze-dried samples were ground into a fine powder in an electric grinder and used for phytochemical and essential nutrient analyses.

4.2.3 Total chlorophyll and carotenoid concentrations

Total chlorophyll and carotenoid concentrations were determined following the procedure by Chen et al., 2019 with some modifications. Leaf samples were extracted with 3 mL of 80% acetone in an ultra-sonicator for 20 min. The absorbance of the clear supernatant was read in a microplate reader (Synergy H1, BioTek, Winooski, VT) at 663 nm, 645 nm, and 470 nm. The concentrations of total chlorophyll and carotenoids were derived using the following relationships:

$\text{Chl a} = 12.72\ \text{A663} - 2.59\ \text{A645}$

$\text{Chl b} = 22.88\ \text{A645} - 4.567\ \text{A663}$

$\text{Total Chl (a + b)} = 20.3\ \text{A645} + 7.22\ \text{A663}$

$\text{Carotenoids} = (1000\ \text{A470} - 3.27\text{Chl a} - 104\text{Chl b})/229$

4.2.4 Total phenolic concentration and antioxidant capacity

Ground leaf samples (0.04 g) were used to determine the concentration of total phenolic compounds and the antioxidant capacity. Total phenolic compound concentration was determined using a modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). Samples were extracted in 4 mL of 80% (v/v) acetone in ultra-sonicator (Ultrasonic Cleaner, Vevor, CA) for 20 min. The extract (1.5 mL) was kept in a refrigerator for 12 h and was then centrifuged at 10,000 x g for 2 min. A 50 µL of the supernatant was mixed with 135 µL distilled water, 750 µl 10% Folin-Ccalteau reagent (Sigma-Aldrich, St. Louis, MO), and 600 µL 7.5% (w/v) Na_2CO_3. The mixture was vortexed and incubated in a water bath at 45^{o}C for 15 min. The absorbance was read at 765 nm (UV/VIS spectrophotometric (U-1100 Spectrophotometer, Hitachi Ltd, Tokyo Japan). The results were expressed as gallic acid equivalent.

The antioxidant capacity was determined using 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method (Miller and Rice-Evans, 1996; Pennycooke et al. 2005). To 20 mL distilled water, 0.0274 g of ABTS and 0.4 g of MnO_2 were added to produce ABTS radicals. ABTS was then mixed with sample extract or Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxyl acid) standards to start the reaction and the absorbance was measured at 730 nm using a spectrophotometer (U-1100 spectrophotometer, Hitachi Ltd, Tokyo, Japan). The results were expressed as the Trolox equivalent.

4.2.5 Individual phenolic compounds

Phenolic compounds from lettuce samples (0.1 g) were extracted using 10 mL of 70% MeOH on an orbital shaker overnight at 4^{o}C in the dark. The extracts were centrifuged (5810R, Brinkman Instruments Inc., Westbury, NY) at 3690 rpm for 30 min. The procedure was repeated twice with 10 mL of 70% MeOH. The pooled supernatant was filtered with qualitative filter paper

(11.0 μm, Global Life Science Solution USA LLC, Marlborough, MA). A 2 mL of the filtered extract was dried in a vacuum dryer for up to 4 h. The residue was redissolved in 1mL of 70% MeOH and the sample was passed through 0.22 μm syringe filter (MilliporeSigma, Burlington, MA) before the high-performance liquid chromatography (HPLC) analyses. Phenolic compounds including gallic acid, caffeic acid, luteolin-7-glucoside, quercetin-3-glucoside, and apigenin-3-glucoside were quantified using HPLC (Shimadzu HPLC, Kyoto, Japan). Phenolic compounds were separated using a reverse phase Waters C18 column (250mm L x 4.6 mm D, Waters, Milford, MA) at 32℃ at an elution rate of 0.8 mL/min. The mobile phase A consisted of formic acid: deionized water (5:95 v/v) and the mobile phase B consisted of formic acid: methanol (5:95 v/v). The gradient was set as follows: 0 to 5 min: 10% B; 5 to 25 min: 40% B; 25 to 41 min: 70% B; 41 to 55 min: 100% B; 55 to 65 min: 0% B. The overall procedure followed was according to Woolley et al. (2019).

4.2.6 Essential nutrients

Freeze-dried ground leaf samples (0.15 g) were used to determine the total carbon and nitrogen concentrations using TruSpec CN instrument with LECO TruSpec CN combustion analyzer. Other essential nutrients such as phosphorus, potassium, calcium, magnesium, manganese, iron, copper, and zinc were analyzed using an ICP spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd., Australia) according to Gieseking et al. (1935). Protein concentrations in the leaves were derived from the leaf nitrogen concentration on dry weight basis according to Milton and Dintzis (1981)

4.2.7 Statistical analysis

Treatment differences with regard to growth characteristics, phytochemical and essential nutrients were analyzed using one-way ANOVA (SAS 9.4, NC and XLSTAT, Addinsoft, New

York, NY). The pairwise comparisons of means were performed using the Duncan's multiple range test at $p < 0.05$, 0.01 and 0.001.

4.3 Results and Discussion

4.3.1 Growth characteristics

Lettuce varieties ('New Red Fire' and 'Two Star') were grown in a growth chambers with a background lighting of approximately 190 μmol/m^2/s and UV-A treatments were started 6 days prior to harvest. LEDs slightly increased the PAR (approximately 192 μmol/m^2/s), and the highest UV-A irradiance was without any lens and the lowest was with the focus lens (Table 4.1) Growth characteristics of lettuce varieties were measured after 3 days and 6 days of supplemental UV-A treatment (Table 4.2). There were no significant changes in most of the growth characteristics including shoot fresh weight, leaf area, and the number of leaves in response to the UV-A treatments. However, shoot dry weights in 'New Red Fire' and in 'Two Star' were suppressed after 6 days of UV treatments. In addition, modifying UV-A LEDs with lenses further depressed the dry shoot biomass in 'Two Star' lettuce even after 3 days of UV treatment. The largest reduction was observed in dry shoot biomass (42% decrease) after 6 days of UV treatment with 5° lens (focus lens) compared to the control plants. Similar observations were made in a previous study using photo-selective films on red leaf lettuce grown in tunnels where UV radiation had a negative impact on its dry matter accumulation (Tsormpatsidis et al., 2008). The impact of UV-A on plant growth and plant functions is complex as it is affected by many factors including genotype, environmental factors, and perhaps, more importantly, the spectral balance of radiation. Thus, plant growth response is highly variable, as suggested by many studies, with some showing positive impacts (Tezuka et al.1994; Brazaitytė et al., 2015) while others showing negative impacts (Kataria et al 2013; Krizek et al., 1997; Krizek et al. 1998; Cooley et al., 2001). Also, there are many reports that show no impact due to UV-A (Jeon et al., 2018). For example, a study by Li and Kubota (2009) on lettuce showed that supplemental UV-A did not have an impact on many growth characteristics, including leaf fresh weight and dry weight, leaf number, and leaf size.

4.3.2 Total chlorophyll and carotenoid concentrations

After exposing plants to UV-A LED for 3 days, leaf chlorophyll concentration increased in ‘New Red Fire’ while longer exposure (6 days) did not result in higher chlorophyll concentration in these plants (Fig. 4.1). Also, LED with 5^o lens (focus lens) reduced the chlorophyll concentration in the leaves relative to the plants under LED without lenses, but the chlorophyll concentration with UV-A LED lenses was similar to the control plants. However, in ‘Two Star’ all the UV-A treatments increased the concentration of chlorophyll after 3 or 6 days of treatment. With regard carotenoids, their accumulation in ‘New Red Fire’ in response to the UV-A treatments was similar to that of chlorophyll accumulation, in that LED without lens produced the largest increase in the carotenoid concentration only after 3 days of treatment (Fig. 4.1). Similarly, response of ‘Two Star’ in the accumulation of carotenoids to UV-A treatments parallels the response of chlorophyll accumulation in that all the UV-A treatments for 3 or 6 days enhanced the carotenoid accumulation in the leaves. These results support the finding of Tezuka et al. (1994), who found that UV-A radiation can enhance chlorophyll content in radish (*Raphanus sativus* L. cv. Akamaru). In contrast, a study by Li and Kubota (2009) showed that supplemental UV-A had no impact on either chlorophyll or carotenoid contents in the leaves of lettuce.

4.3.3 Total phenolic concentration and antioxidant capacity

The total phenolic concentrations in the leaves of ‘New Red Fire’ were reduced by 6 days of supplemental UV-A treatments (Fig. 4.2). The reduction in concentration ranged from 24% to 51% under LEDs compared to the control plants. Similarly, in ‘Two Star’, all the supplemental

UV-A treatments decreased the amount of total phenolic compounds with 3 or 6 days of treatment. On the one hand, the response of antioxidant capacity was delayed, in that 3 days of supplemental UV-A treatment did not affect the antioxidant capacity in both varieties. On the other hand, 6 days of all the supplemental UV-A treatments depressed the antioxidant capacity in both varieties. These results support the findings of Li and Kubota (2009), who examined the effects of supplemental UV-A in a growth chamber study with a background fluorescent lighting (300 $\mu mol/m^2/s$) and found that the total phenolic compound accumulation was not affected by the supplemental UV-A. Similar observations were made on basil (*Ocimum basilicum* L.) grown under supplemental UV-A where the total phenolic compounds and antioxidant capacity were reduced in purple-leaf basil (*Ocimum basilicum* L. cv. Dark Opal) while in green-leaf basil (*Ocimum basilicum* L. cv. Sweet Genovese) they were higher in response to UV-A (Vaštakaitė et al., 2015). In microgreens, supplemental UV-A improved the concentration of total phenolic compounds along with other antioxidant compounds (Brazaitytė, et al., 2015). On the one hand, similar observations were made in dropwort (*Oenanthe stolonifera*) grown under supplemental UV-A LEDs (Jeon et al., 2018). On the other hand, in our greenhouse study, we observed an increase in the amount of total phenolic compounds and the antioxidant capacity only in red leaf lettuce but not in green leaf lettuce (Chapter 3). The variability in UV-A response may depend on many factors including the levels of PAR and UV-B radiation, species, variety, and the experimental conditions (Krizek, 2004). Considering the inconsistency of UV-A response to the accumulation of total phenolic compounds, Verdaguer et al. (2017) has suggested that individual phenolic compounds should be the focus, instead of the total phenolic compounds, in determining the UV response in plants.

4.3.4 Individual phenolic compounds

Accumulation of gallic acid and caffeic acid was monitored after 3 and 6 days of UV treatments in both lettuce varieties. In control plants, the amount of these phenolic acids decreased with age in ‘Two Star’ lettuce (Table 4.3). After 6 days of supplemental UV-A treatment (with scatter lens) of ‘Two Star’ lettuce, only gallic acid increased significantly (approximately by 74%) while no significant differences were observed in caffeic acid in response to any supplemental UV-A treatments. However, supplemental UV-A treatment (with scatter lens) for 3 days suppressed the accumulation of both gallic acid and caffeic acid in the leaves of ‘Two Star’ lettuce. Also, there was no impact of supplemental UV-A treatments on these phenolic acids in ‘New Red Fire’ lettuce (data not presented). With regard to flavonoids, overall, the UV-A treatments suppressed the accumulations of luteolin-7-glucoside, quercetin-3-glucoside, and apigenin-3-glucoside in the leaves of ‘New Red Fire’ in response to 6 days of all the supplemental UV-A treatments with one exception (Fig. 4.3). The exception was the UV-A LED with 5° lens (focus lens) where amounts of luteolin-7-glucoside and quercetin-3-glucoside in the leaves were similar to those in the control plants. Thus, the results suggest that the distribution of UV-A irradiance on the canopy may have marginal impact in the accumulation of phenolic compounds in lettuce.

Although previous studies have found that supplemental UV-A can produce a positive impact on the accumulation of flavonoids (Jeon et al., 2018), the role of PAR has to considered, especially in the accumulation of flavonoids in plants, because PAR has been shown to have a stronger response in inducing the accumulation of flavonoids, which have a role in protecting plants against UV radiation (Krizek, 2004; Morales et al., 2010). This is supported by our greenhouse study, where we supplemented the solar radiation with UV-A and UV-B (Chapter 3). Supplemental UV-A increased the accumulation of a number of flavonoids including luteolin-7-

glucoside, quercetin-3-glucoside, and apigenin-3-glucoside in red leaf ‘New Red Fire’ and, similarly, increased the levels of chlorogenic acid and luteolin-7-glucoside in green leaf ‘Two Star’. This is perhaps due to higher PAR found in the greenhouse (719 μmol/m^2/s) than in a typical growth chamber. A balance between PAR and UV radiation determines the response of plants to UV-A and UV-B, and lower PAR levels are likely to suppress the positive response of UV, not only in growth and but also in the accumulation of phenolic compounds (Krizek 2004: Verdaguer et al., 2017). PAR, especially blue light, along with UV-A has been shown to play an important role in the synthesis of leaf pigments and, thus, in photosynthesis and overall plant growth (Krizek, 2004). Furthermore, there are a number of other factors, such as environmental conditions and plant stresses, that may also impact the responses of plants to UV radiation (Verdaguer et al., 2017). Thus, it is important to use some caution in comparing UV studies conducted in growth chambers, greenhouses, and under the field conditions. The results from this study suggest that the PAR levels in growth chambers need to be high in order to elicit positive response from supplemental UV-A.

4.3.5 Essential nutritional compounds

Supplemental UV-A LED produced an impact on the accumulation of many essential nutrients in both lettuce varieties. Protein is an important nutrient, because it is deficient in human diet in many parts of the world, and it is a major challenge to provide adequate levels in most developing countries. Longer exposure of both varieties of lettuce (6 days) increased the protein concentration in the leaves (Table 4.4 and Fig.4. 4). Supplemental UV-A treatment for 6 days enhanced the amount of protein by approximately 31% in the leaves of ‘New Red Fire’ and by around 48% in the leaves of ‘Two Star’ lettuce. However, modification UV-A irradiance by lenses lowered the protein accumulation in both lettuce varieties. Similar results were observed in radish

(*Raphanus sativus* L. cv. Akamaru) where soluble protein levels increased in response to UV-A radiation (Tezuka et al., 1994)

Longer exposure of ‘New Red Fire’ to supplemental UV-A LED (6 days) resulted in higher accumulation of many essential nutrients including phosphorus (by 19%), potassium (by 19%), sulfur (by 15%), calcium (19%), and manganese (by 30%) compared to the control plants (Table 4.4). Other essential nutrients examined, including magnesium, iron, copper, manganese, and zinc, were not affected by the UV treatments (data not presented). Similarly, in ‘Two Star’ longer exposure produced an increase in phosphorus (by 50%), potassium (by 42%), calcium (44%), magnesium (38%), copper (by 78%), manganese (by 53%), and zinc (by 29%) (Fig. 4.4). However, accumulation of these nutrients due to supplemental UV-A LED treatments were not significant for 3-day exposure (data not presented). Modification of UV-A irradiance with lenses had a variable impact on the accumulation of minor essential nutrients in both lettuce varieties.

Supplemental UV-A had a strong, positive response in relation to the accumulation of essential nutrients, especially protein, which is largely deficient in the human diet in many parts of the world leading malnutrition and consequent serious health issues. In addition, supplemental UV-A increased the accumulation of many essential nutrients in the leaves of both lettuce varieties. The results are consistent with the common observation that crops grown in the open field are typically nutrient-rich compared to those grown in high tunnels (Zhao et al., 2007; Oh et al., 2011). The reason is that crops grown in the open field are exposed full sun including UV radiation, a major part of which is UV-A. This response was similar to that in our greenhouse study with supplemental UV-A and UV-B in lettuce (Chapter 3). Using supplemental UV-A under growth chamber conditions may be less than ideal for improving the accumulation of phytochemicals in lettuce. However, it can promote the accumulation of many essential nutrients, thus playing a major role in improving the nutritional quality of food in our diet. Furthermore, supplementing PAR with UV-A can be a practical strategy in improving the health-promoting qualities of commonly grown leafy vegetables, such as lettuce, especially under protective growing conditions.

Overall, the results show that PAR plays an important role in the response of plants to UV-A. Low PAR levels, common in growth chambers, may induce an inhibitory response of low accumulation of many phenolic compounds in lettuce. Thus, it is important to have adequate PAR levels in growth chambers to simulate the conditions found in the open field where PAR levels are high. Although UV-A LEDs suppressed the phytochemical accumulation in lettuce, they did produce a positive impact on the accumulation of essential nutrients including protein in red leaf lettuce.

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Figures and Tables

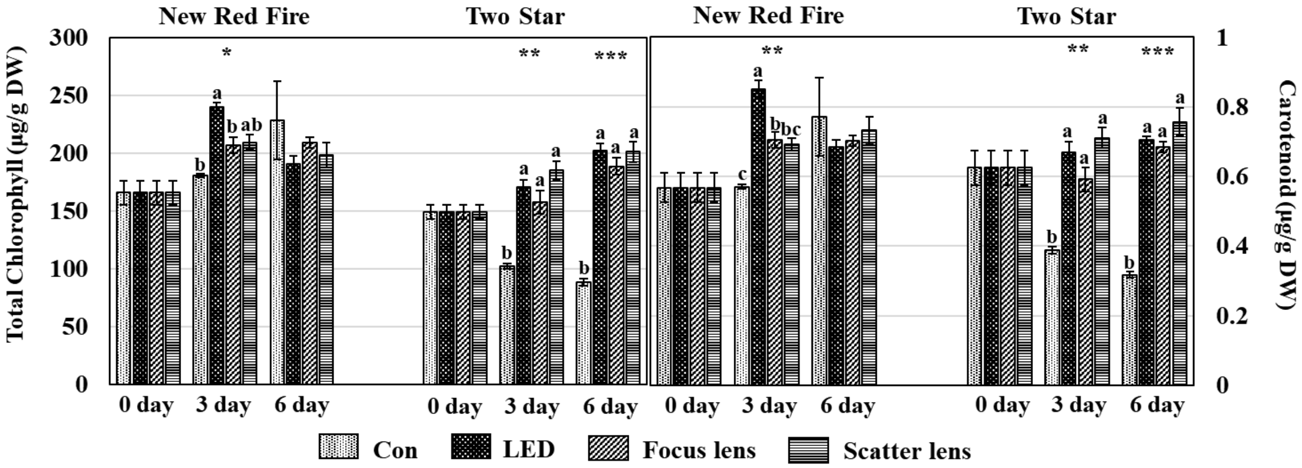

Figure 4. 1. Total chlorophyll and carotenoid concentrations of lettuce varieties, 'New Red Fire' (NRF) and 'Two Star' (TS) subjected to supplemental UV-A radiation. Treatments included LED (without any filter), focus lens (5^{o} lens), and scatter lens. Measurements were made at 0, 3 and 6 days of treatments. Vertical bars indicate standard errors (n=4). Significant differences are presented at * $p < 0.05$, ** $p < 0.01$, *** and $p < 0.001$.

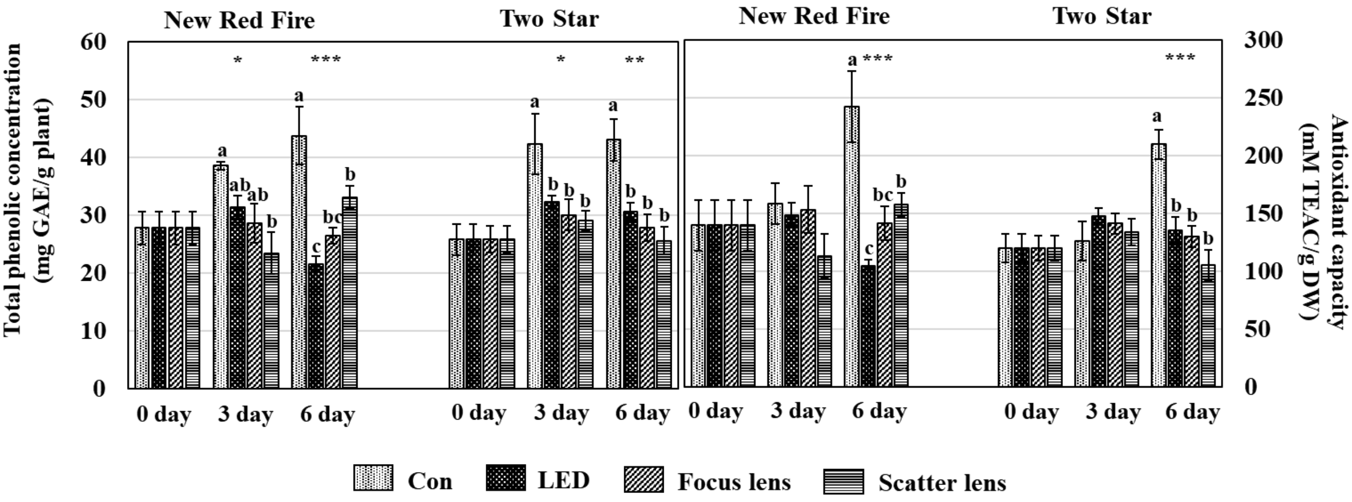

Figure 4. 2. Total phenolic concentration and antioxidant capacity of lettuce varieties, 'New Red Fire' and 'Two Star' subjected to supplemental UV-A radiation. Treatments included LEDs (without any filter), focus lens (5^{o} lens), and scatter lens. Measurement were made at 0, 3, and 6 days of treatment. Vertical bars indicate standard errors (n=4). Significant differences are presented at * p < 0. 05, ** p < 0.01, and *** p < 0.001.

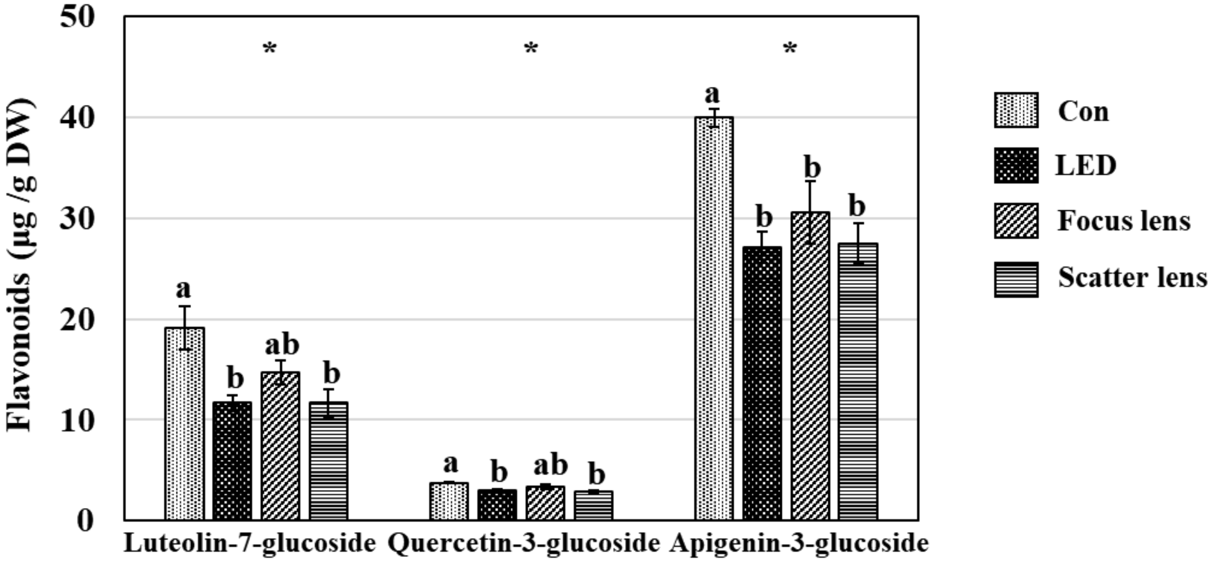

Figure 4. 3. Concentrations of individual phenolic compounds in lettuce variety ‘New Red Fire’ subjected to supplemental UV-A radiation. Treatments included LED (without any filter), focus lens (5^o lens), and scatter lens. Measurements were made after 6 days of treatments. Vertical bars indicate standard errors (n=4). Significant differences are presented at * $p < 0.05$.

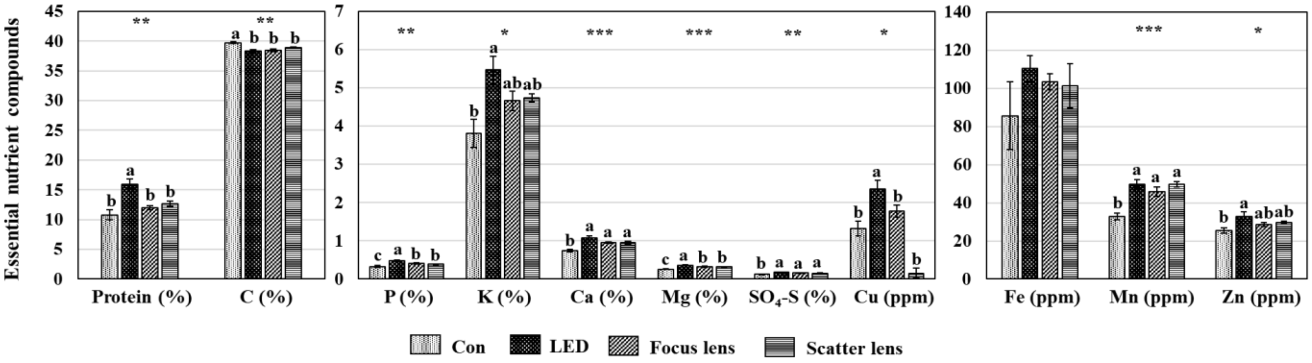

Figure 4. 4. Concentrations of essential nutrients in lettuce variety ‘Two Star’ lettuce subjected to supplemental UV-A radiation. Treatments included LED (without any filter), focus lens (5^o lens), and scatter lens. Measurements were made after 6 days of treatments. Protein concentration was derived from total nitrogen concentration in the leaves. Vertical bars indicate standard errors (n=4). Significant differences are presented at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 4. 1 Average PAR and UV-A levels (with S.E, n = 9) under LEDs (without any filter) and LEDs with focus lens (5° lens) and scatter lens.

	Different Filters			
	Control	LED	Focus lens	Scatter lens
PPFD (μmol/m^2/sec)	190±1.9	192±3.1	197±4.8	192±4.4
UV-A (Watt/m^2)	-	1.37±0.03	0.814±0.1	1.15±0.04

Table 4. 2 Shoot fresh weights, dry weights, leaf area, and leaf number of two lettuce varieties, ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) subjected to supplemental UV-A radiation for 3 or 6 days before harvest. Treatments included LEDs (without any filter), with focus lens (5° lens) and scatter lens (n =4). Measurements were made after 3 and 6 days of treatments.

		New Red Fire				**Two Star**			
		Shoot		Leaf area (cm^2)	Number of leaves	Shoot		Leaf area (cm^2)	Number of leaves
		Fresh weight (g)	Dry weight (g)			Fresh weight (g)	Dry weight (g)		
0 day		43.3	2.8	1056.8	12.7	40.2	3.8	779.32	16
3 day	Control	53.7	3.8	1198.3	14	48.5	5.7 a	941.94	18
	LED	61.5	3.5	1492.3	16	58.6	4.9 ab	1093.97	16.7
	Focus lens	54.7	3.2	1327.7	15	50.8	4.1 b	959.24	16.3
	Scatter lens	56.3	2.8	1246.3	14	55.4	4.4 b	1034.40	15.3
	Significance	ns	ns	ns	ns	ns	*	ns	ns
6 day	Control	62.5	5.1 a	1554.7	15.7	54.5	7.7 a	1027.27	19.7
	LED	61.7	3.5 b	1478.7	14.8	60.3	5.1 b	1201.00	18.5
	Focus lens	59.0	3.4 b	1493.3	15	49.6	4.3 c	974.61	17.3
	Scatter lens	67.6	3.9 b	1636.0	16	56.0	4.4 c	1077.79	16.5
	Significance	ns	**	ns	ns	ns	***	ns	ns

Significant differences are presented at * $p < 0. 05$, ** $p < 0.01$, and *** $p < 0.001$.

NS stands for no significant difference.

Table 4. 3 Concentrations of individual phenolic compounds in lettuce variety, 'Two Star' subjected to supplemental UV-A radiation. Treatments included LED (without any filter), focus lens (5^o lens), and scatter lens. Measurements were made after 3 and 6 days of treatments.

	Different filter study in green lettuce			
	3 days after UV treatments		6 days after UV treatments	
	Gallic acid (µg /g DW)	Caffeic acid (µg /g DW)	Gallic acid (µg /g DW)	Caffeic acid (µg /g DW)
Control	10.24 ab	51.32 a	4.49 b	33.07
LED	14.99 a	40.45 ab	5.07 b	21.24
Focus lens	12.57 ab	43.05 ab	3.93 b	20.61
Scatter lens	6.74 b	33.17 b	7.85 a	17.75
Significance	*	**	*	ns

Significant differences are presented at * $p < 0.05$, ** $p < 0.01$.

NS stands for no significant difference.

Table 4. 4 Concentrations of essential nutrients in lettuce variety ‘New Red Fire’ subjected to subjected to supplemental UV-A radiation. Treatments included LED (without any filter), focus lens (5^o lens), and scatter lens. Measurements were made after 3 and 6 days of treatments. Protein concentrations were derived from total nitrogen in the leaves.

	Light source	Protein (%)	C (%)	P (%)	K (%)	Ca (%)	SO_4-S (%)	Mn (ppm)
3 day	Control	13.25	37.93	0.48 b	5.78 b	0.81	0.166 b	68.08
	LED	15.75	37.31	0.56 ab	6.76 ab	0.86	0.203 ab	70.78
	Focus lens	16.33	36.88	0.65 a	7.35 a	0.95	0.237 a	70.23
	Scatter lens	15.52	36.73	0.61 a	7.11 a	0.96	0.216 a	71.1
	Significance	ns	ns	*	*	ns	*	ns
6 day	Control	14.42 c	38.42 a	0.52 c	6.10 b	0.87 b	0.19 b	59.3 b
	LED	18.92 a	36.86 b	0.62 a	7.26 a	1.04 a	0.22 a	77.1 a
	Focus lens	16.81 b	37.07 b	0.58 b	7.16 a	0.94 ab	0.21 ab	70.6 ab
	Scatter lens	16.81 b	37.34 b	0.55 bc	7.08 a	0.96 ab	0.21 ab	84.1 a
	Significance	***	***	***	**	*	*	*

Significant differences are presented at * $p < 0.\ 05$, ** $p < 0.01$, and *** $p < 0.001$.

Ns stands for no significant difference.

Chapter 5 - Selective Spectral Blocking of Solar Radiation in High Tunnels by Poly Covers: Their Impact on Health-promoting Phytochemicals and Essential Nutrients in Lettuce and Tomato

Abstract

Both visible and UV radiation have a major role in plant growth and development and overall metabolism, including secondary metabolism, which is important for the accumulation of health-promoting phytochemicals in plants. The primary focus of this study was to determine the role of solar UV radiation on the nutritional quality of lettuce (*Lactuca sativa* L., cv. red leaf 'New Red Fire' and green leaf 'Two Star) and tomato (*Solanum lycopersicum* L., cv. BHN-589) grown in high tunnels. Parts of the solar spectrum were blocked using photo-selective poly covers. Treatments included commonly used standard poly, luminescence poly, clear poly, UV blocking poly, removal of standard poly 2 weeks prior to harvest, and standard poly + 55% shade cloth. All the poly covers reduced the PAR levels in the high tunnels, and the largest reduction was by the standard poly + shade cloth, which reduced the PAR by approximately by 48%. Clear poly allowed the maximum UV-A and UV-B radiation, while standard poly allowed around only 16% of solar UV-A and UV-B. Clear poly, which allowed higher percentage of solar UV-A (60.5%) and UV-B (65%), increased the total phenolic concentration and the antioxidant capacity in red leaf lettuce. It also increased the accumulation of flavonoids, including quercetin-3-glucoside, luteolin-7-glucoside, and apigenin-3-gucosude in red leaf lettuce, compared to the standard poly. Removing the standard poly 2 weeks prior to harvest in red leaf lettuce produced the largest increase the accumulation of quercetin-3-glucoside, and it also resulted in an increase in luteolin-7-glucoside and apigenin-3-glucoside. Clear poly increased many essential nutrients, including protein, magnesium, and sulfur in green leaf lettuce. Overall, the standard poly + shade cloth treatment, which had low PAR, UV-A and UV-B, had a negative impact on the accumulation of most of the phenolic compounds. The poly cover treatments did not have any impact on the accumulation of phenolic compounds in green leaf lettuce and tomato fruits. The results show that clear poly can enhance the accumulation of many phenolic compounds in red leaf lettuce and essential nutrients in green leaf lettuce. Also, removal of the standard poly 2 weeks prior to harvest in red leaf lettuce improved the accumulation of many phenolic compounds, suggesting that UV radiation is essential to boost the nutritional quality of lettuce. Thus, removal of the standard poly 2 weeks prior to harvest (simulating movable tunnels) allows the benefits of high tunnel production but with the added advantage of improving the nutritional quality of lettuce.

Keywords: poly cover, spectra quality, phytochemicals, essential nutrients, high tunnel.

5.1 Introduction

High tunnel production of horticultural crops is increasingly becoming popular in the U.S.A., and is already an important plant production system worldwide, especially in Asia. Growers prefer high tunnel production of vegetable and fruit crops, because it is a protective form of agriculture, where crops can be protected from harsh environmental conditions (Carey et al., 2009; Knewtson et al., 2010). In addition, the crops do better and produce higher yields with better aesthetic qualities compared to those grown in open field. The majority of crops grown in high tunnels in the U.S.A. are tomato and lettuce, which are warm and cool-season vegetables, respectively. They are popular vegetable crops and are rich in not only essential nutrients but also in a vast array of health-promoting phytochemicals, including numerous phenolic acids, flavonoids, and carotenoids (Kotíková et al., 2011; Kim et al., 2016; Beecher 1998; Oh et al., 2011). Many phytochemicals are as important as essential nutrients and are particularly known to reduce the risk of numerous chronic and degenerative diseases, including heart disease, cancer, arthritis, and dementia and other neurological diseases (Block et al., 1992; Prior and Cao. 2000; Müller and Krawinkel, 2005; Tulchinsky, 2010). Thus, consumption of fruits and vegetables as part of our daily diet, can promote good health (USDA, 2020). However, our diet, both in the U.S.A. and globally, is inadequate in meeting the daily requirements of essential nutrients and, especially, health-promoting phytochemicals, leading to malnutrition and, thus, serious health issues (Heber 2004). Therefore, it is important to develop strategies to improve the nutritional quality of food in our diet.

Although high-tunnel production can result in increased biomass or yield with greater aesthetic appeal to the consumers, plants grown in high tunnels tend to be deficient in nutritional quality, especially in health-promoting phytochemicals (Oh et al. 2011; Wooley et al., 2019).

Crops grown in high tunnels, especially leafy vegetables, accumulate fewer phenolic compounds and other antioxidants compared to those grown in open field (Zhao et al., 2007; Oh et al., 2011). Although high tunnels are passive structures meant to protect crops against extreme environmental elements, they can alter both the intensity and spectral characteristics of light entering the tunnel. Both intensity and quality of light are important for photosynthesis, light mediated morphogenesis, and the accumulation of nutrients and secondary metabolites (phytochemicals). The standard poly cover used in commercial high tunnels is typically treated to protect against solar UV radiation so as to improve its structural stability and durability. Thus, typically, high tunnels block UV and alter spectral quality of light that plants receive. Many studies have suggested that the reason for lower nutritional quality, especially with regard to phytochemicals, is due to the altered light characteristics that exist under high tunnels, namely reduced UV radiation, which is critical for the accumulation of phenolic compounds in plants (Krizek et al. 1998). Thus, using a poly cover that transmits UV would provide a protective environment and the other benefits of the standard tunnel plus the UV radiation to crops needed to enhance their nutritional quality.

In addition to standard poly, growers are trying different poly covers to alter light characteristics, such as luminance or diffuse poly, to improve the crop performance. Diffuse poly can prevent direct sun light and avoid high temperatures in the tunnel, which may reduce plant stress and improve plant growth. Shade cloths are also often used to reduce the temperature and alter the microclimate within the tunnel for better plant growth. There have been studies aimed at determining plant performance by altering solar radiation with various films. However, a systemic approach to identify a poly cover that can produce better nutritional quality is yet to be considered (Krizek et al., 1998; Luthria et al. 2006).

In the present study, we examine various poly covers for the transmission of UV radiation in an effort to identify a poly cover that will produce better nutritional quality in lettuce and tomato. In this study, we grew plants under the standard poly for most of their growing cycle and then, 2 weeks prior to harvest, the standard poly was removed, so that plants were exposed to full sun simulating what can be done with movable tunnels. We hypothesized that this short plant exposure to solar UV during the last 2 weeks of their growing season was sufficient to improve their nutritional quality.

The main objective of this study was to compare the effects of photo-selective poly covers on the nutritional quality with regard to the health-promoting phytochemicals and the essential nutrients in high-tunnel production of lettuce and tomato. The second objective was to characterize the poly covers with regard to their transmission of solar spectrum and identify the best poly covers that improve the nutritional quality of lettuce and tomato.

5.2 Materials and Methods

5.2.1 Plant materials and growth conditions

The field experiments were conducted at the Kansas State Horticulture Research and Extension Center in Olathe, KS, during spring and fall seasons in 2017. The trials included 2 cultivars of lettuce (*Lactuca sativa* L), namely a red leaf ‘New Red Fire’ and a green leaf ‘Two Star’ (Johnny’s Selected Seeds, Winslow, ME, USA) grown in the spring and fall, and one cultivar of tomato (*Solanum lycooersicum* L), namely ‘BHN 589,’ grown in the summer of 2017. The details of the experimental site and growing conditions are given by Gude (2020). The crops were grown in high tunnels (39.6 m long x 3.7 m wide x 2.1 m high) covered by poly covers with different transmittance of solar spectrum. Each tunnel had 6 treatment plots (6.1 m long each) with a buffer zone (2.1 m long) at each end. The treatments included commercially used standard poly (standard) (single-layer-6- mil, rated for 96% PAR transmission, Klerk's Plastic Product Manufacturing, Inc, Richburg, SC, USA), standard poly removal 2 weeks prior to harvest, simulating movable tunnels (movable), diffuse luminance poly (diffuse) -blocked direct infrared radiation (Luminance; Visqueen Building Products, London, UK), clear poly (clear)- no UV inhibitor (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA), UV blocking poly (block)- blocking UV-A and UV-B (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA), shade cloth (55%) + standard poly (shade) -(Sunblocker Knitted Shade, Farm Tek, Dyersville, IA, USA). Lettuce seeds were germinated in 72-cell propagation trays in a greenhouse and after approximately 4 weeks they were transplanted into high tunnels (October 6th, 2017). Each treatment plot consisted of 2 cultivars of lettuce and the treatment plots in each tunnel were assigned randomly to 6 poly cover treatments using a split-plot RCBD with 4 replications.

Tomato seeds were seeded in a commercial potting mix (Eafard 3B, Agawam, MA, USA) contained in a propagation tray (April 17th, 2017). Seedlings (4-6-week-old) were transplanted into high tunnels as described above under each poly treatment (May 3rd, 2017). Each plot had a buffer zone (1.5 m) on both ends so as to prevent any interference from the neighboring treatments. Treatments were assigned using a randomized, complete block design (RCBD) with 4 replications. After 5-7 weeks, shade cloth was added to shade cloth treatment (July 11th, 2017). Tomato plants were grown following the standard commercial practices. Weeds were suppressed by using woven fabric mulch between the plots. Plants were supported using stake and weave trellis. Fruits were harvested when they were fully ripe (August 31st, 2017).

5.2.2 Light and UV measurements

To characterize the spectral transmission of poly covers, radiation measurements were made under each treatment in high tunnels. Photosynthetically active radiation (PAR), UV-A, and

UV-B were measured during mid-day (11 am -1 pm CST) on 4 clear days on July 10, 14, 17, and 23 in 2017. PAR was measured using a Quantum Radiometer (LI-185B, LI-Cor, Inc., Lincoln, NE), and UV-A and UV-B were measured using a Research Radiometer (ILT5000, International Light Technologies, Peabody, MA). Measurements were made at the canopy level at 9 randomly selected sites in each replication.

5.2.3 Lettuce and tomato fruit sampling

When lettuce plants reached marketable size (~4 weeks), they were harvested. [Give exact dates of harvest.] Two to 3 plants from each plot (replication) were randomly selected and their leaves were freeze-dried (HarvestRight, North Salt Lake, Utah, USA) for 2-3 days or until a stable dry weight was reached. Similarly, 3 tomato plants were randomly selected from each plot and 5 to 6 fully ripe fruits from each plant were harvested. Samples were prepared by separating pericarp from each fruit and freeze-drying them until a stable dry weight was reached. Freeze-dried samples of lettuce and tomato were ground in an electric grinder and stored at -20^{o}C for phytochemical and essential nutrient analyses.

5.2.4 Anthocyanin, chlorophyll, and total carotenoids

Anthocyanin concentration in the leaves was determined using the method by Nakata et al. (2013) with some modifications. Ground, freeze-dried lettuce leaf samples were extracted twice with 0.5 mL of extraction buffer (methanol: acetic acid-45:5 v/v). The supernatants were centrifuged at 12,000 x g for 5 min, and then, their absorbance was measured at 530 and 657 nm in a microplate reader (Synergy H1, BioTek, Winooski, VT, USA). Anthocyanin concentration was calculated using the relationship, (Abs530/g D.W.) = [Abs530-(0.25 x Abs657)]x25. Chlorophyll and total carotenoids were extracted according to the methods of Chen et al. (2019) with some modification. Ground, freeze-dried lettuce samples (0.03 g) were extracted with 3 mL of 80% acetone for 25 min in an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT). The absorbance of the extract was measured in a microplate reader at 663, 645, and 470 nm. The concentrations of chlorophyll and carotenoids were obtained by using the following relationships:

Chl a = 12.72 A663 - 2.59 A645

Chl b = 22.88 A645 - 4.567 A663

Total Chl a + b = 20.3 A645 + 7.22 A663

Carotenoids = (1000 A470 - 3.27Chl a - 104Chl b)/229

5.2.5 Total phenolic compounds and antioxidant capacity

Concentrations of total phenolic compounds were determined using the modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). Ground, freeze-dried lettuce leaf (0.04 g) and tomato fruit (0.4 g) samples were extracted with 4 mL of 80% acetone in ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT). The supernatants were kept in the darkness overnight at 4 ^{o}C. The extract was centrifuged at 1000 rpm for 2 min and a 50 μL of the supernatant was mixed with 135 μL of distilled water, 750 μL diluted (1:10) Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 600 μL of 7.5% (w/v) Na_2CO_3. The mixture was vortexed, and the absorbance was read at 765 nm (U-1100 Spectrophotometer, Hitachi Ltd, Tokyo, Japan) The total phenolic concentration was calculated as a gallic acid equivalent (GAE).

Antioxidant capacity was measured by ABTS (aminobenzotriazole) decolorization assay as outlined by Miller and Rice-Evans, 1996, and Pennycooke et al. (2005) with modification as described by Lee et al. (2019). To generate ABTS* radical cations, a 2.5 mM ABTS stock solution was mixed with 0.4 g of MnO_2, an oxidizing agent. The stock solution was continuously stirred for 30 min at room temperature. The ABTS* solution was diluted with 5 mM PBS buffer (pH 7.4) to get an absorbance value of 0.7 (±0.05) at 730 nm. One mL of ABTS* solution was added to 100 μL of sample supernatants and followed by 1 min of reaction time. The absorbance was measured at 730 nm and the antioxidant capacity of samples was determined as the Trolox equivalent.

5.2.6 Individual phenolic compounds

We examined changes in levels of phenolic acids and flavonoid compounds such as gallic acid, chlorogenic acid, chicoric acid, luteolin-7-glucoside, apigenin-3-glucoside, quercetin-3-glucoside, rutin, and kaempferol in lettuce leaves at the time of harvest. The extraction procedure was based on the method described by Lee et al. (2019) with some modifications. Ground, freeze-dried leaf samples (0.15 g) were extracted with 15 mL of 70% aqueous methanol on an orbital shaker for 12 h at 4°C in the dark. Samples were centrifuged at 3690 rpm for 30 min and the supernatant was collected and the residues were washed with methanol twice and centrifuged again. The pooled supernatant was filtered through a filter paper and evaporated in vacufuge (Concentrator 5301, Hamburg, Germany). A 100 μL sample was analyzed using a Shimadzu HPLC (Shimadzu HPLC, Kyoto, Japan) which was equipped with a UV/VIS detector ranging from 190 to 800 nm. A C18 reversed phase column (250 mm L x 4.6 mm D, Waters, Milford, MA, USA) was used to separate phenolic compounds. The elution gradient solution consisted of solvent A (5:95 v/v formic acid: double deionized water) and solvent B (5:95 v/v formic acid: methanol). The profile of gradient rate for solvent B was as follows: 0 to10% for 5 min, 10-40% for 25 min, 40-70% for 26 min, 70-100% for 10 min and return to 0%. The quantification of phenolic compounds was accomplished using Shimadzu LC Solution Software (Shimadzu HPLC, Kyoto, Japan).

5.2.7 Individual carotenoids

Concentrations of carotenoids including lutein, β-carotene, and lycopene were determined in fully ripe tomato fruits. Ground, freeze-dried samples of tomato pericarp (0.3 g) were extracted with an extraction solution (ethanol: hexane, 4:3, v/v) on an orbital shaker for 4 h at 130 rpm. Samples were then centrifuged at 20℃ and 3950 rpm for 30 min. The supernatant was collected and re-extracted with 8 mL hexane repeatedly. The supernatant was then washed with 30 mL distilled water and then with 30 mL of 10% of sodium chloride. Lipid layer in the supernatant was used for HPLC analyses using the HPLC system described above. Carotenoids were separated using a YMC C30 reversed phase column (250 mm L, YMC America, Inc., Allentown, PA, USA). The elution was performed with solvent A (7:3 v/v methanol: MTBE) and solvent B (100% MTBE). The profile of elution gradient for solvent B rate was as follows: 0-10% for 6 min, 10-20% for 16 min, 20-70% for 26 min, 70 to 10% for 36 min. Individual carotenoids were identified and quantified at 450 nm.

5.2.8 Essential nutrient compounds

Ground lettuce leaf and tomato samples (4 replications) were used to measure the concentration of essential nutrients, including protein, carbon, phosphorus, potassium, calcium, magnesium, sulfur, copper, iron, manganese, and zinc. Carbon and nitrogen were quantified using a LECO TrueSpec CN combustion analyzer. Protein concentration was estimated using the method by Milton and Dintzis (1981). The concentrations of other nutrients were determined using an inductively coupled plasma (ICP) spectrometer (Model 720-ES-ICP Optical Emission Spectrometer, Varian, Australia PTY Ltd., Australia). All the concentrations were expressed on a dry weight basis.

5.2.9 Statistical analyses

Treatment differences with regard to growth characteristics, phytochemical and essential nutrients were analyzed using one-way ANOVA (SAS 9.4, NC and XLSTAT, Addinsoft, New York, NY). The pairwise comparisons of means were performed using the Duncan's multiple range test at $p < 0.05$, 0.01 and 0.001.

5.3 Results and Discussion

5.3.1 Spectral transmission characteristics of poly covers

The radiation measurements in high tunnels covered with various poly covers were made on 4 clear days during mid–July in 2017 around noon (11:00 am -1:00 pm CST) (Fig. 5.1). PAR transmission was similar for most of the poly covers, including the standard poly, diffuse (luminescence poly), clear poly, and UV-block, except for the shade treatment (Table 5.1). With shade cloth over standard poly, PAR transmission was reduced in high tunnels. The PAR transmission with this treatment was reduced by more than 48% of the full sun. The movable treatment involved crops grown under standard poly for most of their growing cycle, except for the last 2 weeks before harvest when they were exposed to full sun. The highest radiation (PAR, UV-A and UV-B) intensity was under movable treatments during that period. Among the high tunnels covered with poly covers, clear poly had the highest UV-A and UV-B intensity, and the shade cloth had much lower level of UV radiation in the high tunnels. All the poly covers blocked UV radiation to various degrees. Standard poly allowed 16.2 % of solar UV-A and 15.8% of solar UV-B. Diffuse poly (luminance poly) was effective in blocking both UV-A and UV-B, while UV-block poly was effective in blocking UV-B more so than UV-A.

5.3.2 Anthocyanins, total chlorophyll, and carotenoids

Anthocyanin and total chlorophyll concentrations in lettuce leaves of both red and green leaf lettuce did not change in response to blocking parts of the solar spectrum using poly covers in high-tunnel production (Fig. 5.2). Similarly, total carotenoids in the leaves of the lettuce varieties did not change in response to poly covers in the high tunnels, except in the case diffuse or luminance poly where total carotenoid concentration of leaves in 'New Red Fire' lettuce increased compared to the plants under standard ploy. Krizek et al. (1998) examined the role of UV-A and UV-B on the growth of red leaf lettuce by selectively blocking the solar spectra and found that UV-A and UV-B enhanced the concentration of both anthocyanin and chlorophyll b in the leaves.

5.3.3 Total phenolic concentration and antioxidant capacity

The total phenolic concentration in 'New Red Fire' increased only under clear poly compared to that under standard poly or all other coverings (Fig. 5.3). However, the total phenolic concentration and the antioxidant capacity did not change in response to various poly covers in green leaf 'Two Star' lettuce. Under clear poly, the total phenolic concentration in 'New Red Leaf'' increased by more than 29 % over the standard poly. As clear poly transmits more UV radiation than other poly covers, UV radiation is likely to play a positive role in the accumulation the total phenolic compounds. These results are consistent with previous study on the effect of UV on red leaf lettuce that showed a higher accumulation of phenolic compounds with increasing levels of UV radiation (Garcia-Macias et al., 2007). In red leaf lettuce, UV-blocking poly, which significantly blocks UV-B, also reduced the accumulation of total phenolic compounds, suggesting that UV-B may play an important role in the accumulation of phenolic compounds in 'New Red Fire' lettuce. Similar results were observed in 'New Red Fire' lettuce, where UV-B resulted in accumulation of phenolic compounds (Krizek et al., 1998). Similarly, growing lettuce in open fields, which received the full spectrum of solar radiation including UV, accumulated more phenolic compounds than the ones grown in high tunnels with standard poly, which typically blocks substantial UV radiation (Oh et al., 2011). With reduced PAR and UV radiation, ‘New Red Fire’ lettuce under the shade treatment (high-tunnel) accumulated the least amount of phenolic compounds with lowest antioxidant capacity. With regard to tomato fruits, blocking parts of the solar radiation spectrum with poly covers had no effect either on accumulation of phenolic compounds or the antioxidant capacity (Fig. 5.3). On the contrary, Luthria et al. (2006) found that tomato fruits grown in high tunnels that allowed UV radiation had higher total phenolic compounds. Their study examined different cultivars and the transmission characteristic of UV blocking films, which were different from the current study. These could be reasons for the variable outcomes.

5.3.4 Individual phenolic compounds and carotenoid contents

Phenolic acids and flavonoids were quantified in the leaves of both ‘New Red Fire’ and ‘Two Star’ at the time of harvest using HPLC. The retention times for various phenolic compounds are presented in Table 5.2. There was an impact of various poly covers on the accumulation of flavonoids. Red leaf ‘New Red Fire’ lettuce was impacted more than green leaf ‘Two Star’ lettuce; both clear poly and standard poly removal (movable) prior to harvest produced higher accumulation of luteolin-7-glu in ‘New Red Fire’ lettuce (Fig. 5.4). Removing standard poly 2 weeks prior to harvest produced the highest accumulation of luteolin-7-glu (approximately 71% more) than that in plants under standard poly. Similarly, removal of standard poly prior to harvest and clear poly had a positive impact on the accumulation of apigenin-3-glu and quercetin-3-glu in the leaves of ‘New Red Fire’ lettuce (Fig. 5.4 and Table 5.3). The largest increases in both flavonoids (increases by 62% in apigenin-3-glu and by 50% in quercetin-3-glu) in the leaves of

‘New Red Fire’ lettuce was again in response to removal of standard poly prior to harvest. This is consistent with results from our greenhouse study involving the same lettuce varieties, where we supplemented solar radiation with UV. Supplementing UV-A enhanced the concentration of the above flavonoids in the red leaf ‘New Red Fire’ lettuce (Chapter 3). Similar observations have been made on red leaf lettuce (‘Lollo Russo’); increasing levels of UV radiation increased the accumulation phenolic acids and flavonoids (Garcia-Macias et al., 2007). Many studies comparing the accumulation of health-promoting phytochemicals in leafy vegetables have found that crops grown crops in an open field, which has the benefit of receiving full sun including UV, have higher amounts of phenolic compounds than ones grown in high tunnels (Oh et al. 2011; Zhao et al., 2007). This is further supported by the fact that other poly covers, such as diffuse poly, which transmits similar levels of PAR as clear poly but blocks both UV-A and UV-B radiation, has no impact on the accumulation of flavonoids. On the one hand, UV-block poly allows UV-A radiation but blocks UV-B and, thus, has little impact on the accumulation of these compounds. On the other hand, clear poly and full sun (movable tunnel) provide greater levels of not only UV-A but also UV-B. Clear poly provides the maximum fraction of solar UV-A and UV-B (Table 5.1), as does the exposure of these plants to full sun prior to harvest (movable tunnel). The results suggest that UV radiation plays a dominant role in inducing the accumulation of flavonoids in lettuce. Also, even a short exposure of plants to UV can improve the accumulation of these health-promoting flavonoids. This may have a practical benefit in that growers can grow lettuce (New Red Fire) in high tunnels and expose the plants to full sun for just 2 weeks before harvest to improve the flavonoid content, thereby improving the nutritional quality of the crop. Similar results were observed in a previous study using red lettuce varieties, where antioxidant capacity of lettuce (due to antioxidant enzymes) was enhanced by exposing plants to high light intensity prior to harvest (Hipol et al., 2014).

Contrary to the positive effects of clear poly and standard poly removal, shade treatment suppressed the accumulation of flavonoids, including luteoilin-7-glu, apigenin-3-glu, and quercetin-3-glu and also chlorogenic acid in ‘New Red Fire’ lettuce. This is because of the low fluence of PAR and UV radiation in high tunnels under shade treatment (Table 5.1).

In the case of green leaf ‘Two Star’ lettuce, most of the poly cover treatments in this study did not have any effect on the accumulation of most of the phenolic compounds except the shade treatments, which suppressed the accumulation of luteolin-7-glu and quercetin-3-glu (Fig. 5.4 and Table 5.3). The use of shade fabric, which can reduce the PAR and UV significantly, is likely to have an adverse effect not only on the accumulation of phenolic compounds but also on the biomass accumulation in lettuce (Gude, 2020).

The accumulation of individual carotenoids, including lutein, β-carotene and lycopene, in tomato fruits from plants grown under various poly covers was examined. The results showed no impact of poly covers on the accumulation of these carotenoids (Fig. 5.5). However, in our previous study, supplementing solar radiation with UV in a greenhouse was found to enhance the concentration of carotenoids (lycopene, lutein, and β-carotene) in tomato fruits (Chapter 3).

5.3.5 Essential nutrients

There was an impact of various poly covers on the accumulation of essential nutrients only in the green leaf ‘Two Star’ lettuce but not in red leaf ‘New Red Fire’ lettuce (Fig. 5.6 and Table 5.4). Also, no impact of these treatments on the accumulation of essential nutrients in tomato fruits was observed (Table 5.5). In green leaf ‘Two Star’ lettuce, leaf protein content increased in plants grown under diffuse poly, clear poly, UV-block, and shade treatment compared to those under standard poly. The increase in protein concentration was more than 19% in response to these treatments. Similarly, the accumulation of potassium and magnesium in these plants also increased in response to the above treatments by more than 12% and 16%, respectively, compared those under standard poly. The results that green leaf ‘Two Star’ lettuce responds better to light factors is consistent with our greenhouse studies (Chapter 3) involving supplemental UV. Supplemental UV-A or UV-B increased the accumulation of protein, potassium, magnesium, and zinc in green leaf ‘Two Star’ but not red leaf ‘New Red Fire’ lettuce. In a study comparing the nutritional quality of lettuce crops grown in an open field and high tunnel with standard poly, accumulation of essential nutrients, including protein content in plants, was affected by a high-tunnel environment and the results were variable with regard to nutrients and varieties (Woolley et al., 2019).

Protein deficiency and malnutrition due to micronutrient deficiency are globally a widespread public health crisis (Müller and Krawinkel, 2005, Wu et al., 2014). Therefore, growing food crops rich in nutrients is a practical strategy to combat this widely prevalent public health issue. Controlling the light characteristics in crop production under protective environments is beneficial in the production of nutrient-dense food. The results from this study show that manipulating spectral characteristics can improve the nutritional quality of vegetables, especially those most commonly grown in high tunnels such as lettuce and tomato. Furthermore, the emerging LED technology and the development of new poly covers for high-tunnel crop production can further enhance our ability to manipulate light characteristics, which can benefit not only growers of horticultural food crops and but also consumers.

In summary, standard poly currently used in high tunnels reduces the transmission of PAR, UV-A, and UV-B. Therefore, the crops grown in such tunnels typically have poor nutritional value. Thus, clear poly, which allows more UV-A and UV-B, enhances the nutritional quality of lettuce resulting from higher accumulation of many phenolic compounds. Similar results were observed when a lettuce crop (red leaf) was grown in high tunnels with standard poly until 2 weeks before harvest and followed by the removal of poly covers to expose the crop to full sun. This can also be accomplished by using movable tunnels to expose the crop to full sun 2 weeks prior to harvest to boost the nutritional quality of lettuce. Thus, the results suggest that exposure of a lettuce crop to full sun (containing UV) for a short period of time is enough to make a significant improvement in its nutritional quality.

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Figures and Tables

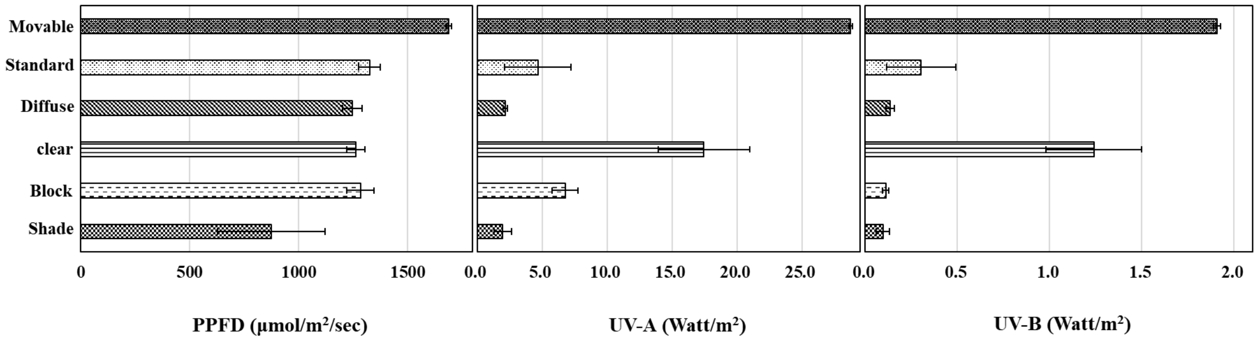

Figure 5. 1. PAR and UV radiation in high tunnels covered with photo-selective poly covers. The measurements were made at the canopy level in mid-July on 4 separate days with clear sky during mid-day (11:00 am-1:00 pm (CST)). Poly covers included standard poly, removal of standard poly 2 weeks prior to harvest (movable), diffuse (luminance) poly, clear poly, UV-block poly, and standard poly + shade cloth. For movable treatment, radiation measurements were made after the standard poly covers were removed. Therefore, it represents radiation of full sun.

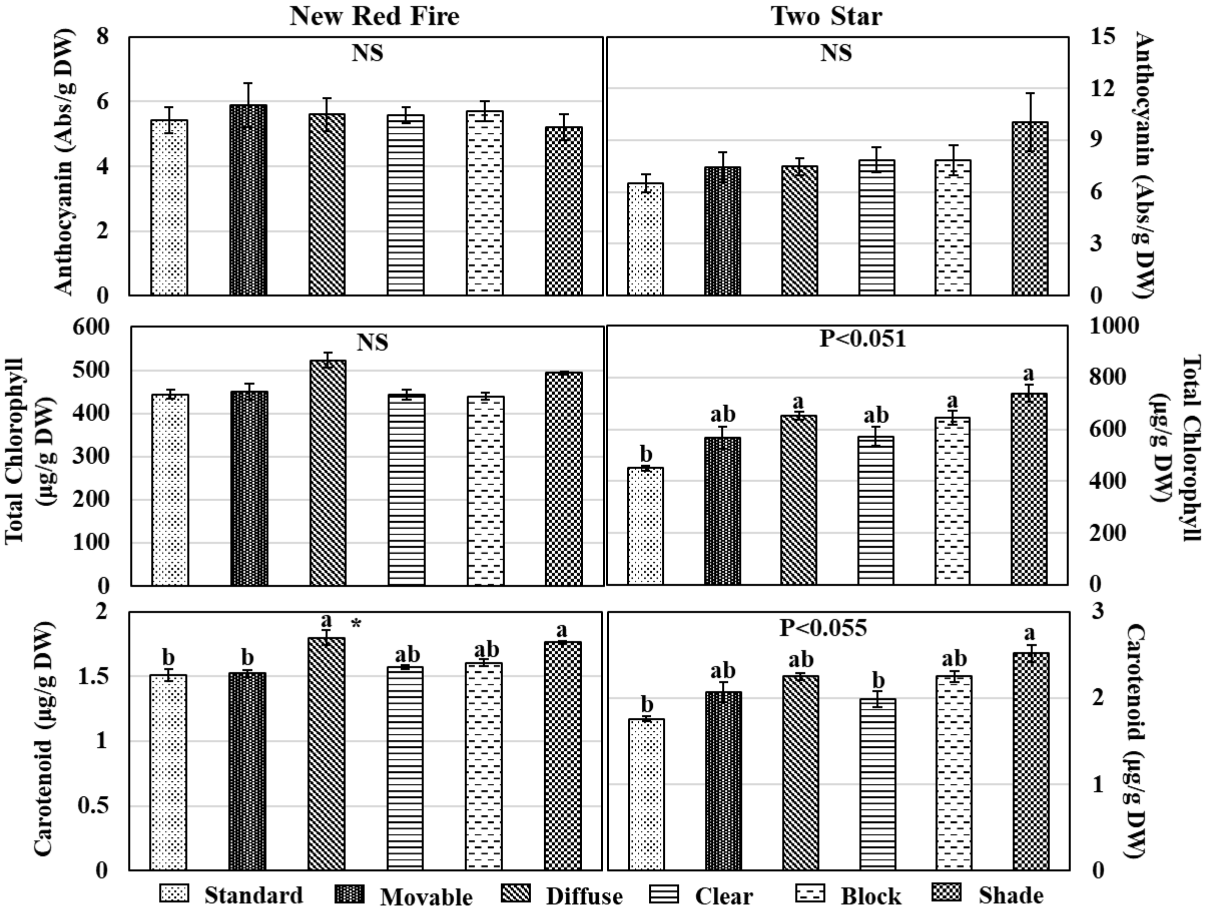

Figure 5. 2. The anthocyanin, the total chlorophyll, and the carotenoid concentrations in leaves of lettuce varieties, ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade). The vertical bars indicate standard errors (n=4). Significant differences at * $p < 0.05$ and marginally statistical difference p-value levels are presented. NS stands for no significant difference.

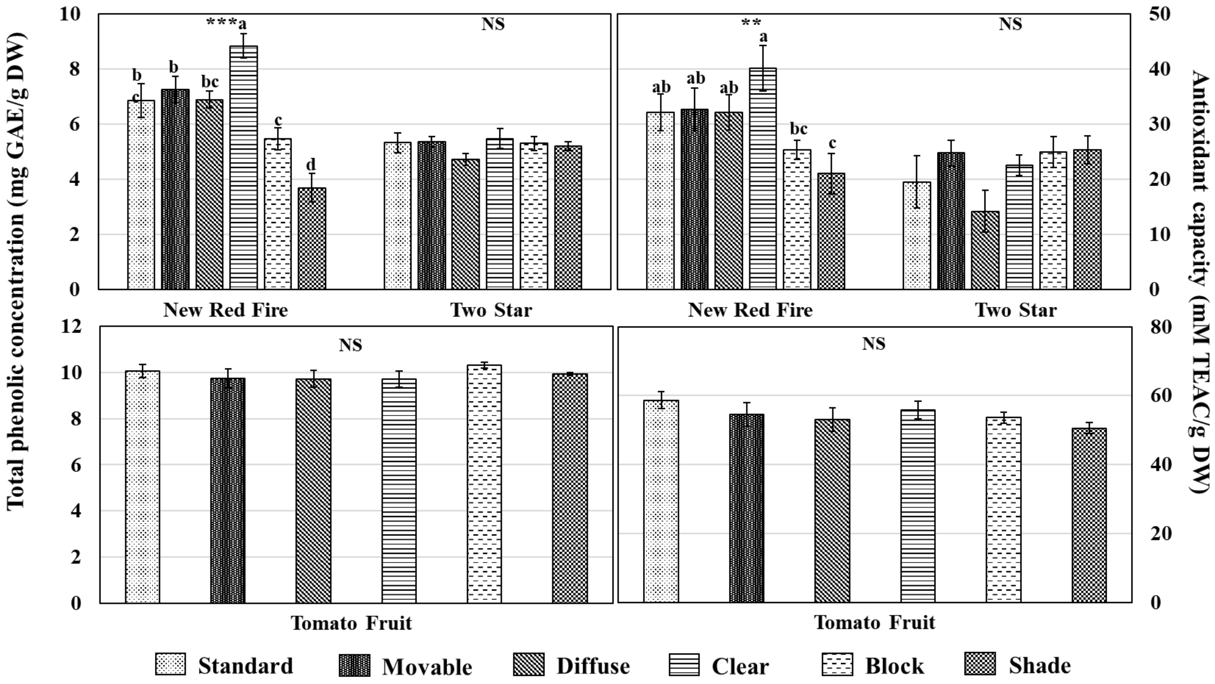

Figure 5. 3. The total phenolic concentration and antioxidant capacity in leaves of lettuce varieties, ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) and tomato fruits (var: ‘BHN-589) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade). The vertical bars indicate standard errors (n=4). Significant differences are presented at ** $p < 0.01$ and *** $p < 0.001$. NS stands for no significant difference.

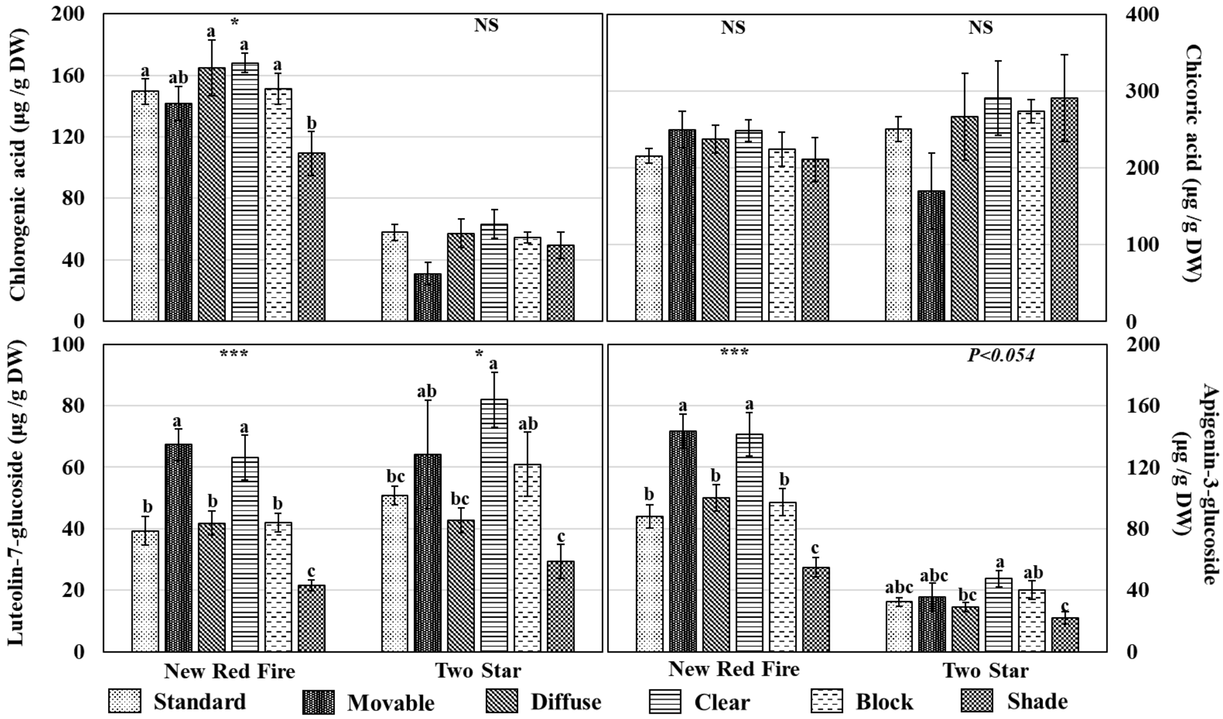

Figure 5. 4. The concentrations of phenolic compounds in leaves of lettuce varieties, ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade). The vertical bars indicate standard errors (n=4). Significant differences at * $p < 0.05$, *** $p < 0.001$, and marginally statistical difference p-value levels are presented. NS stands for no significant difference.

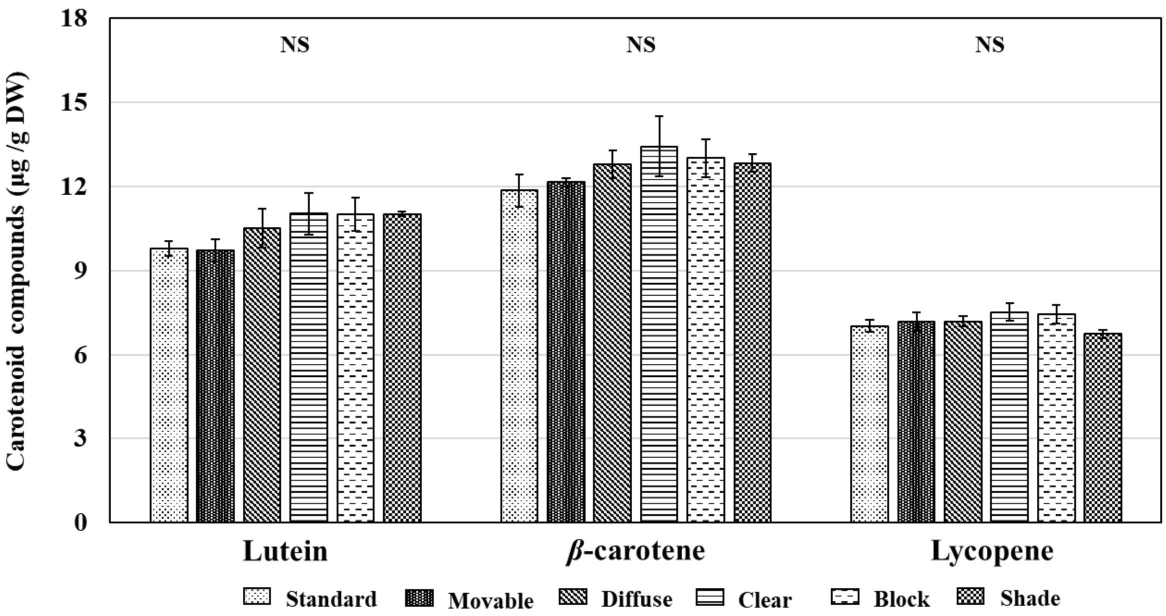

Figure 5. 5. The concentrations of carotenoids in tomato fruits (var: ‘BHN-589) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade). The vertical bars indicate standard errors (n=4). NS stands for no significant difference.

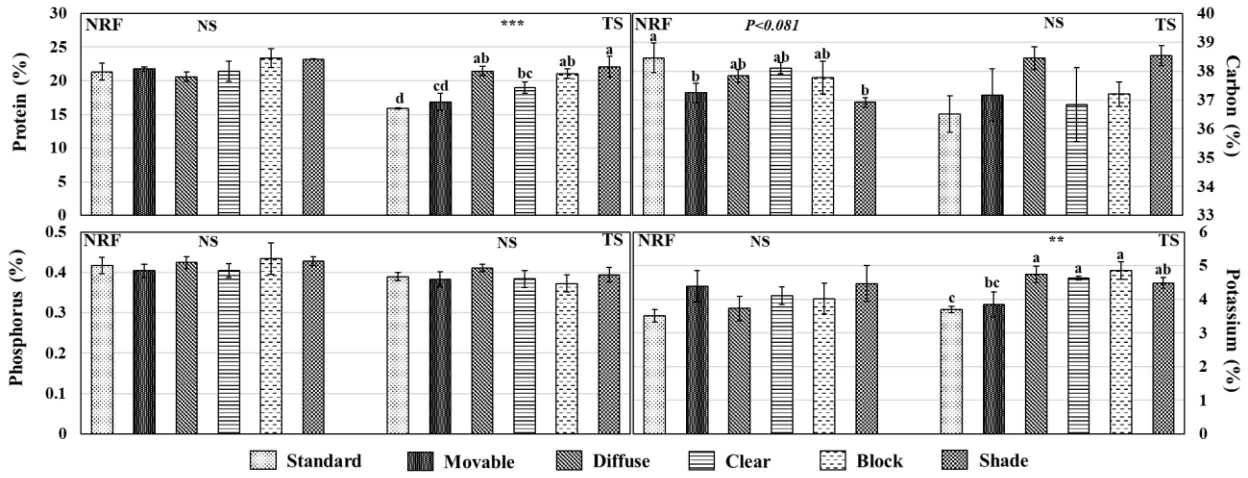

Figure 5. 6. The concentrations of essential nutrients in leaves of lettuce varieties, 'New Red Fire' (NRF) and 'Two Star' (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade). The vertical bars indicate standard errors (n=4). Significant differences ** $p < 0.01$ and *** $p < 0.001$ and marginally statistical difference p-value levels are presented. NS stands for no significant difference.

Table 5. 1 Transmission of radiation under various poly covers. Relative percent transmission is presented.

Poly cover	**Percent relative transmission**		
	PAR	UV-A	UV-B
Movable	78.6[a]-100[b]	16.2[a]-100[b]	15.8[a]-100[b]
Standard	78.6	16.2	15.8
Diffuse	73.8	7.5	7.1
Clear	74.7	60.7	65.0
Block	76.1	23.5	5.8
Shade	51.8	6.8	5.0

[a]Standard poly removal (movable poly)- measurements made when crops were grown under standard poly.
[b]Measurements made when standard poly was removed 2 weeks prior to harvest to expose the crops to full sun and the transmission is set to 100% transmission

Table 5. 2 Retention times for phenolic compounds and carotenoids in HPLC analyses of lettuce varieties, ‘New Red Fire’ (NRF), ‘Two Star’ (TS), and tomato fruits’ (var: ‘BHN-589) at the time of harvest.

Phytochemicals	RT (min)
Phenolic compounds	
Gallic acid	5.5
Chlorogenic acid	19.46
Chicoric acid	30.77
Luteolin-7-glucoside	33.065
Quercetin-3-glucoside	33.407
Rutin	33.67
Apigenin-3-glucoside	34.65
Kaempferol-3-glucoside	35.43
Carotenoids	
Lutein	11.955
β-carotene	16.65
Lycopene	25.533

RT: retention time (in minutes)

Table 5. 3 The concentrations of phenolic compounds in leaves of lettuce varieties, 'New Red Fire' (NRF) and 'Two Star' (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade).

		Phytochemicals compounds (µg /g DW)			
Lettuce		Gallic acid	Quercetin-3-glucoside	Rutin	Kampferol-3-glucoside
New Red Fire	Standard	16.41	8.76 b	2.25	7.11
	Movable	15.96	13.12 a	1.88	6.22
	Diffuse	13.25	9.55 b	2.00	6.92
	Clear	16.38	12.54 a	2.03	6.85
	Block	16.78	8.80 b	1.89	5.62
	Shade	13.12	5.97 c	1.71	5.41
	Significance	NS	***	NS	NS
Two Star	Standard	12.09	5.58	2.65	3.85
	Movable	9.92	6.65	1.76	3.01
	Diffuse	15.72	5.44	2.33	3.91
	Clear	16.25	8.54	2.10	4.25
	Block	13.34	6.38	2.07	3.88
	Shade	13.81	5.23	2.48	4.54
	Significance	NS	NS	NS	NS

Significant differences are presented at *** $p < 0.001$.
NS stands for no significant difference.

Table 5. 4 The concentrations of mineral nutrients in leaves of lettuce varieties ‘New Red Fire’ (NRF) and ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade).

		Mineral compounds						
Lettuce		Ca %	Mg %	SO_4-S %	Cu ppm	Fe ppm	Mn ppm	Zn ppm
New Red Fire	Standard	1.01	0.50	0.24	6.2	512.2	71.3	39.5
	Movable	1.12	0.47	0.25	6.1	786.6	84.2	36.8
	Diffuse	1.03	0.49	0.25	6.1	607.0	74.0	38.7
	Clear	1.11	0.52	0.26	5.6	384.2	62.6	35.4
	Block	1.17	0.55	0.26	5.9	523.8	80.6	40.1
	Shade	1.16	0.54	0.25	5.4	458.5	76.8	37.8
	Significance	NS	NS	NS	NS	NS	NS	NS
Two Star	Standard	1.26	0.36 d	0.23 b	5.7	523.2	67.3	37.0
	Movable	1.35	0.40 cd	0.24 b	5.5	336.0	78.1	33.2
	Diffuse	1.42	0.48 ab	0.28 a	4.8	399.5	71.9	34.5
	Clear	1.34	0.42 bc	0.26 a	5.1	325.2	64.0	33.2
	Block	1.43	0.51 a	0.28 a	5.2	422.4	74.6	36.5
	Shade	1.39	0.50 a	0.28 a	4.3	276.3	65.6	33.8
	Significance	NS	***	**	NS	NS	NS	NS

Significant differences are presented at ** $p < 0.01$ and *** $p < 0.001$.
NS stands for no significant difference.

Table 5. 5 The concentrations of essential nutrients tomato fruits (var: 'BHN-589') at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely standard poly (standard), removal of standard poly 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-block poly (block), and standard poly with shade cloth (shade).

	Essential nutrient compounds for tomato fruits										
	Protein %	C %	P %	K %	Ca %	Mg %	SO_4-S %	Cu ppm	Fe ppm	Mn ppm	Zn ppm
Standard	14.4	37.7	0.3	3.1	0.16	0.1	0.12	8.0	52.0	12.5	19.3
Movable	14.7	37.4	0.3	3.3	0.15	0.2	0.12	7.4	71.5	14.5	19.8
Diffuse	14.7	37.1	0.3	3.3	0.15	0.1	0.20	43.9	68.4	13.1	19.7
Clear	13.8	37.6	0.3	3.2	0.16	0.2	0.12	7.5	52.6	11.9	19.4
Block	15.1	37.4	0.3	3.2	0.17	0.2	0.13	7.5	102.5	14.5	20.7
Shade	16.4	37.1	0.4	3.7	0.12	0.2	0.14	8.1	67.6	12.4	23.9
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS stands for no significant difference.

Chapter 6 - Conclusion

The main objective of these studies was to characterize the role of spectral quality of both visible and UV radiation on the nutritional quality of commonly grown vegetables such as lettuce and tomato. We used supplemental light using LEDs of specific spectral bands (blue, red, and far-red) on red leaf and green leaf lettuce varieties grown in growth chambers. We used supplemental UV-A and UV-B using conventional sources of lighting on lettuce and tomato grown in a greenhouse. Supplemental visible light had an impact on plant growth in lettuce. In both red and green lettuce varieties, supplemental far-red produced larger plants, higher biomass, and larger leaf area akin to a shade-avoidance mechanism. With regard to nutritional quality of lettuce, the response was variable depending on the variety. Supplemental blue radiation increased the accumulation of many phenolic compounds in green leaf lettuce, while supplemental red radiation showed similar responses in red leaf lettuce. Although supplemental far-red radiation produced higher biomass, it had an inhibitory effect on the accumulation of phenolic compounds. None of the supplemental treatments in the visible spectrum enhanced the essential nutrient concentrations in lettuce. Nonetheless, supplemental light in the visible region has a strong influence on enhancing the concentrations of health-promoting phytochemicals and, thus, improving the nutritional quality of lettuce.

Similar to the response to supplemental visible light, supplemental UV-A also increased a number of phenolic compounds in both red and green leaf lettuce. Supplemental UV treatments increased the accumulation of many essential nutrients, including protein and the mineral nutrients phosphorus, potassium, copper, manganese, and zinc. This response occurred in green leaf lettuce more so than in red leaf lettuce. Thus, supplemental UV radiation can increase the nutritional quality by enhancing health-promoting phytochemicals as well as important essential nutrients in lettuce. However, these responses to UV in lettuce can vary depending on the ambient levels of photosynthetic radiation (PAR), in that low PAR, as found in growth chambers, can bring about a negative impact on the accumulation of phytochemicals. Similarly, In the case of tomato, supplemental UV-A and supplemental UV-AB (3 h exposure) can increase the accumulation of many phenolic compounds including caffeic acid, chicoric acid, luteolin-7-glucoside, kaempferol-3-glucoside, and apigenin-3-glucoside in the fully ripe fruits. In addition, supplemental UV, especially UV-A, increased the accumulation of carotenoids, including lutein and β-carotene, which are important for eye health in humans. Supplemental UV-AB had a positive impact on the accumulation of lycopene in fully ripe fruits, which is known to reduce the risk of several types of cancers in humans.

Thus, supplemental UV radiation plays a role in improving nutritional quality in relation the accumulation of health-promoting phytochemicals and, especially, in enhancing the levels of essential nutrients in lettuce and tomato. Plants receive UV from solar radiation, but when crops are grown in protected environments, such as high tunnels, solar radiation is altered. In high tunnels crops receive a low light intensity and low levels of UV radiation compared to the open field. This will likely have a negative impact on the nutritional quality of crops grown in high tunnels. In recent years, an increasing number of horticultural crops are grown in high tunnels because they protect crops from harsh environmental conditions. In addition, crops grown under high tunnels produce more biomass and are aesthetically more appealing to the consumers. However, these crops tend to be less nutritious. This problem can be alleviated if appropriate poly covers that allow greater amount of solar radiation, especially UV, are used to cover the tunnels. The standard poly covers commonly used in high tunnels by growers are treated with UV-blocking agents so that poly covers do not degrade rapidly under full sun. Our results show that use of clear poly can improve the nutritional quality by enhancing the accumulation of many health-promoting phenolic compounds. In addition, removing the standard poly just 2 weeks prior to harvest, and exposing lettuce to full sun, will enhance the nutritional quality of the crop. This again underscores the importance of UV radiation for enhancing the nutritional quality of leafy vegetables. Thus, growers can grow crops in high tunnels with standard poly and still improve the nutritional quality of crops by exposing them to full sun just prior to harvest.