Abstract: Spectral characteristics of solar radiation have a major role in plant growth and development and the 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 effect of spectral characteristics of solar 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 in relation to the accumulation of essential nutrients and phytochemicals. Solar spectrum received by crops was modified using photo-selective poly covers. Treatments included commonly used standard poly, luminescence poly (diffuse poly), clear poly, UV blocking poly, exposure of crops grown under the standard poly to full sun 2 weeks prior to harvest (akin to movable tunnel), and 55% shade cloth on the standard poly. All the poly covers and shade cloth reduced the PAR levels in the high tunnels, and the largest reduction was by the shade cloth, which reduced the solar PAR by approximately 48%. Clear poly allowed the maximum UV-A and UV-B radiation, while standard poly allowed only a small fraction of the solar UV-A and UV-B (between 15.8% and 16.2%). Clear poly, which allowed a higher percentage of solar UV-A (60.5%) and UV-B (65%) than other poly covers, 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-glucoside in red leaf lettuce, compared to the standard poly. Brief exposure of crops grown in high tunnels to full sun prior to harvest produced the largest increase in the accumulation of quercetin-3-glucoside, and it also resulted in an increase in luteolin-7-glucoside and apigenin-3-glucoside in red leaf lettuce. Thus, clear poly and brief exposure of red leaf lettuce to the full sun, which can increase UV exposure to the plants, produced a positive impact on its nutritional quality. In contrast, shade cloth which allowed the lowest levels of solar PAR, UV-A and UV-B relative to the other poly covers had a negative impact on the accumulation of the phenolic compounds in red leaf lettuce. However, in green leaf lettuce, luminesce poly, clear poly, UV-block poly, and shade treatments increased the accumulation of many essential nutrients, including protein, magnesium, and sulfur in green leaf lettuce compared to the standard poly. Poly cover treatments including shade treatment did not affect the accumulation of either carotenoids (lutein, β-carotene, and lycopene) or essential nutrients in mature tomato fruits. The results show that clear poly cover can enhance the accumulation of many phenolic compounds in red leaf lettuce, as does the brief exposure of the crop to the full sun prior to harvest. Thus, UV radiation plays an important role in the accumulation of phenolic compounds in red leaf lettuce while the overall spectral quality of solar radiation has a significant influence on the accumulation of essential nutrients in green leaf lettuce.
Keywords: essential nutrients; high tunnel; phytochemicals; poly cover; spectral quality

1. Introduction

High tunnel production of horticultural crops is increasingly becoming popular in the U.S., and is already an important plant production system worldwide, especially in Asia [1,2]. 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 [3,4]. In addition, crops do better and produce higher yields with better aesthetic qualities compared to those grown in open fields. The majority of crops grown in high tunnels in the U.S. 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 [5–8]. 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, dementia, and other neurological diseases [9–12]. Thus, consumption of fruits and vegetables as part of our daily diet can promote good health [13]. However, our diet, both in the U.S. and globally, is inadequate in meeting the daily requirements of essential nutrients and health-promoting phytochemicals, leading to malnutrition and, thus, to serious health issues [14]. 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 [7,15]. Crops grown in high tunnels, especially leafy vegetables, accumulate low amounts of phenolic compounds and other antioxidants compared to those grown in open fields [7,16]. 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 [17]. Thus, manipulation of light characteristics can be used to alter the growth, yield, and morphogenesis of crops grown under protective environmental conditions [18]. The standard poly cover used in commercial high tunnels is typically treated to protect it against solar UV radiation which can have a harmful impact on the poly cover structural stability and durability. Thus, typically, high tunnels with the standard poly block most of the solar UV radiation and alter the spectral quality of light that plants receive. One of the major reasons attributed for the lower nutritional quality in crops grown in high tunnels, especially with regard to phytochemicals, is the altered light characteristics that exist under high tunnels, namely reduced UV radiation, which can hamper the accumulation of phenolic compounds in plants [17–19]. Thus, using a poly cover that transmits UV will not only provide a protective environment and the other benefits of a standard tunnel to crops but also potentially improve their nutritional quality.

In addition to the standard poly, growers are trying different poly covers to alter light characteristics, such as luminance or diffuse poly, to improve crop performance [20]. Luminescence poly can prevent direct sunlight and avoid high temperatures in the tunnel, thus reducing plant stress and improving plant growth. Shade cloths are also often used to reduce the temperature and alter the microclimate within the tunnel and thus, the plant growth and other plant responses [21]. Additionally, there have been studies aimed at determining plant performance by altering solar radiation with various films [19,22]. Various poly covers have been used to determine the accumulation of many phytochemicals during various maturity stages and postharvest stages in lettuce and tomato [23]. However, little attention has been focused on identifying poly covers that can produce crops with better nutritional quality in high tunnel production systems. Although the focus of
many previous studies has been to examine the impact of light on the accumulation of health-promoting phytochemicals [16,22,24], very little is known about the accumulation of essential nutrients including protein and mineral elements in food crops as influenced by the spectral quality of light. Therefore, one of the objectives of the present study was to assess the impact of photo-selective poly covers and the possible role of UV on the overall nutritional quality as determined by key health-promoting phytochemicals and essential mineral nutrients in lettuce and tomato fruits. Additionally in the present study, we examine various poly covers for their transmission of solar radiation in an effort to identify a poly cover that will produce better nutritional quality in lettuce and tomato. Previous studies [7,15,16] have shown that crops grown in open fields tend to have high nutritional quality compared to those grown in high tunnels. However, it is not known if short exposure of crops grown in traditional high tunnels to the full sun would improve their nutritional quality. In the present study, we will expose lettuce and tomato crops grown in high tunnels to full sun (simulating movable tunnel) for 2 weeks just prior to harvest. As these crops are grown under protective conditions for most of their growing season, their growth and marketability are less likely to be affected by the brief exposure to the full sun just before harvest. However, it is not known if brief exposure of crops to full sun has any impact on the accumulation of health-promoting phytochemicals and essential nutrients.

The main objective of this study was to compare the effects of photo-selective poly covers on the overall nutritional quality including key health-promoting phytochemicals and the essential nutrients in high-tunnel production of lettuce and tomato. The study also focuses on characterizing various poly covers with regard to their transmission of the solar spectrum in order to identify the best poly covers or the strategies to improve the nutritional quality of lettuce and tomato in high tunnel production systems.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The field experiments were conducted at the Kansas State Horticulture Research and Extension Center in Olathe, Kansas (lat. 38.885359° W) during 2017–18. The soil is Chase silt loam. The study included two 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) and one cultivar of tomato (Solanum lycopersicum L.), namely ‘BHN 589.’ The crops were grown in three seasons (spring, summer, and fall) during 2017–2018. The present study is part of a larger multi-year field study examining the impact of poly covers on the growth and nutritional quality of lettuce and tomato during pre-and postharvest stages in high tunnel production systems. The details of the experimental setup and growing conditions are described elsewhere [23]. The crops were grown in high tunnels (39.6 m long × 3.7 m wide × 2.1 m high) covered by poly covers with different transmittance of solar spectrum. There were 4 high tunnels representing blocks and each tunnel had six treatment plots (each 6.1 m long) with a buffer zone (2.1 m long) at each end. The treatments included (1) commercially used standard poly (standard) (single-layer-6-mil, rated for 92% PAR transmission, Klerk’s Plastic Product Manufacturing, Inc, Richburg, SC, USA), (2) standard poly removal 2 weeks prior to harvest, simulating movable tunnels (movable), (3) luminance poly (diffuse poly)-rated for blocking direct infrared radiation (Luminance; Visqueen Building Products, London, UK), (4) clear poly (clear)- with no UV inhibitor (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA), (5) UV blocking poly (block)- rated for blocking UV-A and UV-B (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA), and (6) 55% shade cloth on the standard poly (shade) (Sunblocker Knitted Shade, Farm Tek, Dyersville, IA, USA). Temperatures under the tunnels were recorded continuously at 30 min intervals during the growing seasons using temperature probes (2/treatment; EL-USG-1; Laser Electronics, Erie, PA, USA). As the base of each tunnel was left open (0.5 m high from the ground) without poly covers for free air circulation, the temperatures under high tunnels did not vary much among treatments and also from the
temperature of the open field (Table 1) More detailed data on microclimate conditions within the tunnels and crop performance are presented elsewhere [23,25]. Lettuce seeds were germinated in 72-cell propagation trays in a greenhouse, and after approximately four weeks, they were transplanted into high tunnels in staggered rows (26.7 cm within the rows × 26.7 cm between rows). Each treatment plot consisted of 2 cultivars of lettuce and the treatment plots in each tunnel were assigned randomly to six poly cover treatments using a CRBD with 4 replications. Plants were harvested when they reached marketable size (4 weeks post-transplant in spring and 8 weeks post-transplant in fall).

Table 1. Average maximum and minimum temperatures in high tunnels with various poly covers during growing seasons in Olathe, KS.

| Season | Temperature °C | Max 2 | DOF 1 | Min 2 | DOF |
|--------|----------------|-------|-------|-------|-----|
| Spring | 25.60 ± 1.30   | 0.98 ± 0.82 | 10.5 ± 1.15 | 0.83 ± 0.70 |
| Summer | 29.80 ± 0.77   | 0.61 ± 0.46 | 19.50 ± 0.69 | 0.55 ± 0.34 |
| Fall   | 17.75 ± 1.00   | 0.73 ± 0.67 | 10.50 ± 1.15 | 0.83 ± 0.70 |

1 DOF is the average temperature deviation in the tunnel from the open field (±S.E.) measured during the growing seasons (2017–2019); and 2 Average temperatures (±S.E.) in the tunnels. Temperatures were recorded in 30 min increments during the growing seasons.

Tomato seeds were seeded in a commercial potting mix (Eafard 3B, Agawam, MA, USA) contained in a propagation tray. Seedlings (4–6 weeks old) were transplanted into high tunnels under each poly treatment. Treatments were assigned using a randomized complete block design with four replications. The experimental layout was similar to that in lettuce trials. The trial consisted of four high tunnels, each representing a block containing all poly cover and shade cloth treatments. Two beds ran lengthwise in each tunnel (39.6 m long × 0.61 m wide) with buffer zones (1.5 m) at each end of the plot. The seedlings were transplanted after approximately 6 weeks in a single row (45 cm between plants with 1 m between rows). After 5–7 weeks of transplanting, a shade cloth was added to the shade cloth treatment. Tomato plants were grown following the standard growing practices used in high tunnel production [25]. Plants were drip-irrigated as needed and weeds were suppressed in the plots by using woven fabric mulch. Plants were supported using a stake and weave trellis system. Fruits were harvested when they were fully ripe (fruit skin color > 50% red).

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, USA), and UV-A and UV-B were measured using a Research Radiometer (ILT5000, International Light Technologies, Peabody, MA, USA) according to the manufacturer’s guidelines. Measurements were made at the canopy level at 9 randomly selected sites in each replication.

2.3. Lettuce and Tomato Fruit Sampling

When lettuce plants reached marketable size (~4 weeks after transplanting), they were harvested. Two to three plants from each plot (replication) were randomly selected and their leaves were harvested and freeze-dried (HarvestRight, North Salt Lake, UT, USA) for two to three days or until a stable dry weight was reached. Similarly, three tomato plants were randomly selected from each plot and five to six fully ripe fruits from each plant were harvested. Samples were prepared by separating pericarp from each fruit and
freeze-drying until a stable dry weight was reached. Freeze-dried samples of lettuce and tomato were ground in an electric grinder and stored at −20 °C for further analyses.

2.4. Anthocyanin, Chlorophyll and Total Carotenoids

Anthocyanin concentration in the leaves was determined using the method by Nakata et al. [26] with some modifications. Ground, freeze-dried lettuce leaf samples (0.02 g) were extracted twice with 0.5 mL of extraction buffer (methanol: acetic acid-45:5 v/v). The supernatants were centrifuged at 12,000 × 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 × Abs657)] × 25. In addition, 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, USA). 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, and carotenoids = (1000 A470 − 3.27Chl a − 104Chl b)/229.

2.5. Total Phenolic Compounds and Antioxidant Capacity

Concentration of total phenolic compounds was determined using the modified Folin-Ciocalteu method [27]. Ground, freeze-dried lettuce leaf (0.04 g) and tomato pericarp (0.4 g) samples were extracted with 4 mL of 80% acetone in an ultrasonic processor (Vibra-Cell, Sonics and Materials Inc., Danbury, CT, USA). The supernatants were kept in the darkness overnight at 4 °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) Na2CO3. 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, [28] and Pennycooke et al. [29] 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 MnO2, an oxidizing agent. The stock solution was continuously stirred for 30 min at room temperature. The ABTS* solution was diluted with a 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.

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. [24] 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 a 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 with a range from 190 to 800 nm. A C18 reversed-phase column (250 mm L × 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: 0–10% 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).

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°C 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, 70–10% for 36 min. Individual carotenoids were identified and quantified at 450 nm.

2.8. Essential Nutrients

Ground lettuce leaf and tomato pericarp samples (four 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 [30]. 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.

2.9. Statistical Analyses

Treatment differences with regard to growth characteristics, phytochemical and essential nutrients concentrations were analyzed using two-way ANOVA (SAS 9.4, NC and XLSTAT, Addinsoft, New York, NY, USA). The field trial was conducted on CRBD with four replications and the treatment effects were determined with regard to phytochemicals and essential nutrients with no significant blocking effect. The pairwise comparisons of means were performed using Duncan’s multiple range test at $p < 0.05$, 0.01, and 0.001.

3. Results and Discussion

3.1. Spectral Transmission Characteristics of Poly Covers and Shade Cloth

The radiation measurements in high tunnels covered with various poly covers were made on four clear days during mid–July in 2017 around noon (11:00 a.m.–1:00 p.m. CST) (Figure 1). The movable treatment involved crops grown under the standard poly for most of their growing cycle, except for the last two weeks before harvest when they were exposed to full sun. Radiation measurements for the movable treatment represent the full sun radiation as these measurements were made after the poly covers were removed from the high tunnels.
PAR transmission in high tunnels was reduced under all the poly covers, the reduction in PAR was similar among all the poly covers including the standard poly. However, the largest reduction in PAR was with the shade cloth treatment (55% shade cloth used over standard poly), which was more than 48% of full sun. Similarly, all the poly covers also reduced the transmission of both UV-A and UV-B to various degrees; however, the use of shade cloth was the most effective treatment in blocking UV radiation. Additionally, among all the treatments examined, shade cloth was most effective in blocking the overall solar radiation in high tunnels. Luminance poly was effective in blocking both solar UV-A and UV-B, while UV-block poly (Dura Film Super 4) was effective in blocking UV-B more so than UV-A. Clear poly transmitted a greater amount of both solar UV-A and UV-B (>60 of solar UV) compared to other poly covers while the standard poly allowed 16.2% of solar UV-A and 15.8% of solar UV-B.

### 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 modifying the solar spectrum using the poly covers and shade cloth in high-tunnel production (Figure 2). Similarly, the concentration of total carotenoids in the leaves of lettuce was not affected by the poly covers or shade cloth in the high tunnels, except in the case of luminance poly where the total carotenoid concentration of leaves increased in the red leaf lettuce compared to the plants under the standard poly. Krizek et al. [19] 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. However, in the present study, although clear poly which transmitted higher levels of UV-A and UV-B in high tunnels (Figure 1) relative to other poly covers, did not have any effect on the accumulation of both chlorophyll and anthocyanin in the leaves. This may suggest that even clear poly with the highest transmission of UV-A and UV-B may not allow enough UV to have a positive impact on the accumulation of these pigments.

**Figure 1.** PAR and UV radiation in high tunnels covered with photo-selective poly covers. Poly covers included the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminance poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). Line bars indicate S.D. For movable treatment, radiation measurements were made after the standard poly covers were removed. Therefore, it represents radiation from the full sun.
Figure 2. The anthocyanin, the total chlorophyll, and the carotenoid concentrations in leaves of lettuce varieties, red leaf ‘New Red Fire’ (NRF) and green leaf ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnels covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminance poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). The vertical bars indicate standard errors (n = 4). Letters on the bars indicate significant difference, and bars with same letters are not significantly different. Significant differences at $p < 0.05$ (*) and marginal statistical difference $p$-value levels are presented. NS stands for no significant difference.

3.3. Total Phenolic Concentration and Antioxidant Capacity

The total phenolic concentration in the red leaf lettuce increased only under clear poly compared to that under the standard poly or all other coverings (Figure 3). However, the total phenolic concentration and the antioxidant capacity were not affected by any poly covers or shade cloth in green leaf ‘Two Star’ lettuce. Under clear poly, the total phenolic concentration in the red leaf lettuce increased by more than 29% over the standard poly. UV radiation is likely to play a positive role in the accumulation of the total phenolic compounds as clear poly transmits more UV radiation than other poly covers or shade cloth. Similar results were observed in red leaf lettuce where the higher accumulation of phenolic compounds occurred in response to increasing levels of UV radiation [31]. The role of UV is illustrated by the fact that all the poly covers block solar UV to various degrees while clear poly allows more solar UV than others and thus, has a positive impact on the accumulation of phenolic compounds. In fact, shade cloth was more effective in blocking both UV-A and UV-B, which actually suppressed the accumulation of total phenolic compounds and the antioxidant capacity in red leaf lettuce compared to the standard poly. (Figures 1 and 3). It produced the lowest accumulation of phenolic compounds and the weakest antioxidant capacity in red leaf lettuce. Results by Krizek et al. [19] further confirm the role of UV in the accumulation of phenolic compounds where they found that exposure of ‘New Red Fire’ lettuce to UV-B resulted in the accumulation of phenolic compounds in the plants. In addition, growing lettuce in open fields, which received the full spectrum of solar radiation including UV, accumulated more phenolic compounds than the crops grown in high tunnels with the standard poly which typically reduces the substantial amounts of solar UV radiation [7].
With regard to tomato fruits, modifying the solar radiation spectrum with the poly covers or shade cloth had no effect either on the accumulation of total phenolic compounds or the antioxidant capacity (Figure 3). On the contrary, Luthria et al. [22] found that tomato fruits grown in high tunnels that allowed UV radiation had higher total phenolic compounds. However, these results were with different tomato varieties using UV-blocking films with different transmission characteristics from the ones used in this study. In addition, in our study, all the poly covers reduced the transmission of both solar UV-A and UV-B radiation including clear poly which reduced the UV transmission by approximately 40%. Tomato, unlike lettuce, may need a higher level of UV for a positive response with regard to the accumulation of total phenolic compounds.

3.4. Individual Phenolic Compounds and Carotenoids

Accumulation of phenolic acids and flavonoids in the leaves of both red leaf and green leaf lettuce at the time of harvest is presented in Figure 4 and Table 2. The red leaf lettuce was more responsive to spectral blocking with regard to the accumulation of these phytochemicals than the green leaf lettuce. Consistently, movable treatment and clear poly increased the accumulation of quercetin-3-glucoside, luteolin-7-glucoside, and apigenin-3-glucoside in the red leaf lettuce relative to the standard poly while clear poly was effective in improving the accumulation of luteolin-7-glucoside in the green leaf lettuce. It is important to note that although PAR transmission under all the poly covers was similar while the transmission of UV-A and UV-B was variable under these poly covers (Figure 1). The fact that clear poly allows greater transmission of UV-A and UV-B compared to other poly covers points to the positive role UV may play in the accumulation of phenolic compounds. Similarly, movable treatment which exposed the plants to full sun (including UV radiation) albeit for a short period of time (two weeks) resulted in higher accumulation of phenolic compounds in the red leaf lettuce. The largest increases were observed in...
luteolin-7-glucoside (71%), apigenin-3-glucoside (62%), and quercetin-3-glucoside (50%) compared to the standard poly in response to brief exposure of the red leaf lettuce to full sun. This further suggests that UV plays an important role in the accumulation of phenolic compounds in lettuce. This is consistent with the results from our previous greenhouse study on the same lettuce varieties, which showed that supplementing solar radiation with UV-A enhanced the concentration of many flavonoids in the red leaf 'New Red Fire' lettuce [32]. Additionally, similar observations have been made on red leaf lettuce (Lollo Russo) where increasing levels of UV radiation increased the accumulation of phenolic acids and flavonoids in the leaves [31] and also, in lettuce grown in an open field as opposed to high tunnels [7,16].

![Figure 4](image_url)

**Figure 4.** The concentrations of phenolic compounds in leaves of lettuce varieties, red leaf ‘New Red Fire’ (NRF) and green leaf ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnels covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminescence poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). The vertical bars indicate standard errors (n = 4). Letters on the bars indicate significant difference, and bars with same letters are not significantly different. Significant differences at p < 0.05 (*), p < 0.001 (**), and marginal statistical difference p-value levels are presented. NS stands for no significant difference.

The importance of UV in inducing the accumulation of phenolic compounds is supported by the fact that luminescence poly and other poly covers, which transmit similar levels of PAR as clear poly but block both UV-A and UV-B radiation, had no impact on the accumulation of flavonoids. Clear poly and brief exposure of crop to the full sun (akin to movable tunnel) provide greater levels of both UV-A and UV-B than other poly covers resulting in a higher accumulation of phenolic compounds in the red leaf lettuce. In contrast, UV-blocking poly cover which allowed some UV-A radiation but was effective in blocking UV-B had little impact on the accumulation of these compounds suggesting that UV-B may have a greater role in the accumulation of flavonoids in red leaf lettuce.
Table 2. The concentrations of phenolic compounds in leaves of lettuce varieties, red leaf ‘New Red Fire’ (NRF) and green leaf ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnel covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminance poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). NS stands for no significant difference.

|                   | Phytochemicals Compounds (µg/g DW) | Lettuce | Gallic Acid | Chicoric Acid | Rutin | Kaempferol-3-Glucoside |
|-------------------|------------------------------------|---------|-------------|---------------|-------|------------------------|
|                   |                                    | NRF     | 16.41       | 215.47        | 2.25  | 7.11                   |
|                   |                                    | NRF     | 15.96       | 249.74        | 1.88  | 6.22                   |
|                   |                                    | NRF     | 13.25       | 237.39        | 2.00  | 6.92                   |
|                   |                                    | NRF     | 16.38       | 248.30        | 2.03  | 6.85                   |
|                   |                                    | NRF     | 16.78       | 224.11        | 1.89  | 5.62                   |
|                   |                                    | NRF     | 13.12       | 210.91        | 1.71  | 5.41                   |
|                   |                                    | NRF     | NS          | NS            | NS    | NS                     |
|                   |                                    | Standard| 12.09       | 250.07        | 2.65  | 3.85                   |
|                   |                                    | Standard| 9.92        | 169.81        | 1.76  | 3.01                   |
|                   |                                    | Standard| 15.72       | 266.56        | 2.33  | 3.91                   |
|                   |                                    | Standard| 16.25       | 290.83        | 2.10  | 4.25                   |
|                   |                                    | Standard| 13.34       | 273.27        | 2.07  | 3.88                   |
|                   |                                    | Standard| 13.81       | 290.46        | 2.48  | 4.54                   |
|                   |                                    | Standard| NS          | NS            | NS    | NS                     |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 16.64       | 229.83        | 2.15  | 5.85                   |
|                   |                                    | TS      | 14.52       | 229.83        | 2.15  | 5.85                   |

The finding that even a short exposure of plants to UV (movable) can improve the accumulation of these health-promoting flavonoids can potentially lead to 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 their nutritional quality of the crop. Similar results were observed in a previous study using red leaf lettuce varieties, where the antioxidant capacity of lettuce (due to antioxidant enzymes) was enhanced by exposing plants to high light intensity prior to harvest [33].

Contrary to the positive effects of clear poly and the brief exposure of crops to full sun, shade treatment suppressed the accumulation of flavonoids, including luteolin-7-glucoside, apigenin-3-glucoside, and quercetin-3-glucoside, and also chlorogenic acid in red leaf lettuce. This is perhaps due to the low flux of PAR and UV radiation in high tunnels under the shade treatment (Figure 1). 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 [23].

In both red and green leaf lettuce, the accumulation of some phenolic compounds including gallic acid, chicoric acid, rutin, and kaempferol-3-glucoside was not affected by altering the solar radiation spectrum either by poly covers or shade treatment (Table 2). Gude [23] examined the accumulation of phenolic compounds in red and green leaf lettuce during postharvest storage and found that rutin concentration was higher in red leaf lettuce grown in fall under clear and UV-blocking poly covers than under the standard poly cover but was not affected in the green leaf lettuce in spring and fall grown crops.

Similarly, the accumulation of individual carotenoids, including lutein, β-carotene, and lycopene, in mature tomato fruits was not affected by poly covers or shade cloth (Figure 5). This may be due to the reduced overall flux of PAR and UV in the high tunnels due to the high tunnel coverings. However, in contrast, in a previous greenhouse study on tomato, Lee et al. [32] showed that actually supplementing solar radiation with UV enhanced the accumulation of carotenoids including lycopene, lutein, and β-carotene in mature tomato fruits.
3.5. Essential Nutrients

Accumulation of essential nutrients including nitrogen (protein), carbon, phosphorus, potassium, calcium, iron, magnesium, sulfur, copper, manganese, and zinc in the leaves of lettuce and tomato fruits as affected by altering the solar spectrum is presented in Figure 6 and Tables 3 and 4. Accumulation of essential nutrients in the leaves of red leaf lettuce and tomato fruits was not affected by the poly covers or shade treatment. However, in green leaf lettuce, concentrations of protein, potassium, magnesium, and sulfur increased consistently under clear poly, luminescence poly, UV-blocking poly, and shade cloth compared to the standard poly (Figure 6). The increase in protein concentration was more than 19% in response to these treatments. Similarly, the accumulation of potassium and magnesium in the leaves also increased in response to the above treatments by more than 12% and 16%, respectively, compared to those under the standard poly. It is interesting to note that all the poly covers including shade cloth had a positive impact on the accumulation of these essential nutrients but not on the accumulation of phenolic compounds in the green leaf lettuce relative to the standard poly. Overall, the results show that modification of the solar radiation spectrum in high tunnels has a strong influence on the nutritional quality of lettuce. Its effects, however, were variable depending on the crop and variety in that it affected primarily the accumulation of phenolic compounds in the red leaf lettuce while it had a similar impact on the accumulation of some of the essential nutrients in the green leaf lettuce. However, such modification of spectral characteristics of solar radiation in high tunnels was found to have no significant impact on the nutritional quality of tomato fruits (Table 4).
Potassium and magnesium in the leaves also increased in response to the above treatments by more than 12% and 16%, respectively, compared to those under the standard poly. It is interesting to note that all the poly covers including shade cloth had a positive impact on the accumulation of these essential nutrients but not on the accumulation of phenolic compounds in the green leaf lettuce relative to the standard poly. Overall, the results show that modification of the solar radiation spectrum in high tunnels has a strong influence on the nutritional quality of lettuce. Its effects, however, were variable depending on the crop and variety in that it affected primarily the accumulation of phenolic compounds in the red leaf lettuce while it had a similar impact on the accumulation of some of the essential nutrients in the green leaf lettuce. However, such modification of spectral characteristics of solar radiation in high tunnels was found to have no significant impact on the nutritional quality of tomato fruits (Table 4).

Figure 6. The concentrations of essential nutrients in leaves of lettuce varieties, red leaf ‘New Red Fire’ (NRF) and green leaf ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnels covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminescence poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). The vertical bars indicate standard errors (n = 4). Letters on the bars indicate significant difference, and bars with same letters are not significantly different. Significant differences $p < 0.01 (**)$ and $p < 0.001 (***)$. NS stands for no significant difference.

Table 3. The concentrations of mineral nutrients in leaves of lettuce varieties red, leaf ‘New Red Fire’ (NRF) and green leaf ‘Two Star’ (TS) at the time of harvest. The plants were grown in high tunnels covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminance poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). NS stands for no significant difference.

| Lettuce       | Ca % | Cu ppm | Fe ppm | Mn ppm | Zn ppm |
|---------------|------|--------|--------|--------|--------|
| New Red Fire  |      |        |        |        |        |
| Standard      | 1.01 | 6.2    | 512.2  | 71.3   | 39.5   |
| Movable       | 1.12 | 6.1    | 786.6  | 84.2   | 36.8   |
| Diffuse       | 1.03 | 6.1    | 607.0  | 74.0   | 38.7   |
| Clear         | 1.11 | 5.6    | 384.2  | 62.6   | 35.4   |
| Block         | 1.17 | 5.9    | 523.8  | 80.6   | 40.1   |
| Shade         | 1.16 | 5.4    | 458.5  | 76.8   | 37.8   |
| Significance  | NS   | NS     | NS     | NS     | NS     |
| Two Star      |      |        |        |        |        |
| Standard      | 1.26 | 5.7    | 523.2  | 67.3   | 37.0   |
| Movable       | 1.35 | 5.5    | 336.0  | 78.1   | 33.2   |
| Diffuse       | 1.42 | 4.8    | 399.5  | 71.9   | 34.5   |
| Clear         | 1.34 | 5.1    | 325.2  | 64.0   | 33.2   |
| Block         | 1.43 | 5.2    | 422.4  | 74.6   | 36.5   |
| Shade         | 1.39 | 4.3    | 276.3  | 65.6   | 33.8   |
| Significance  | NS   | NS     | NS     | NS     | NS     |

Thus, 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 lettuce, a crop commonly grown in high tunnels. 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 but also ultimately the consumers.
Table 4. The concentrations of essential nutrients tomato fruits (var: ‘BHN-589’) at the time of harvest. The plants were grown in high tunnels covered with different poly covers namely the standard poly (standard), removal of the standard poly 2 weeks prior to harvest (movable), luminescence poly (diffuse), clear poly (clear), UV-blocking poly (block), and the standard poly + shade cloth (shade). NS stands for no significant difference.

| Essential Nutrients for Tomato Fruits | Protein % | C % | P % | K % | Ca % | Mg % | SO₄-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     |

Furthermore, it is also important to consider crop performance including growth, yield, and aesthetic quality under these poly covers in addition to improving the nutritional quality. The present study is part of a larger study where, in addition to nutritional quality, crop performance of these crops as influenced by these poly covers were also investigated (see Gude [23]). Overall, the results show that crop growth, yield, and aesthetic qualities in both crops grown under various poly covers were comparable to crops grown under the standard poly cover. There was no difference in yield of lettuce grown under clear poly, UV-block and luminescence poly or standard poly. In addition, sensory analyses showed that lettuce (red leaf) developed higher color intensity under clear poly and the moveable treatment than under the standard poly (Gude et al. [25]). Similarly, there was no significant difference in biomass accumulation or marketable yield of tomato crops grown under different poly covers. In summary, the results showed the poly covers used in this study (except shade treatment) did not have a significant negative impact on the growth or yield of lettuce and tomato.

4. Conclusions

In summary, the standard poly currently used in high tunnels reduces the transmission of PAR, UV-A, and UV-B. Crops grown in traditional high tunnels with the standard poly typically have poor nutritional value because of reduced solar radiation including UV. However, clear poly, which allows more UV-A and UV-B than the standard poly, enhances the nutritional quality of lettuce resulting in a higher accumulation of many phenolic compounds in red leaf lettuce. Similar results were observed with regard to the accumulation of phenolic compounds when the standard poly cover was removed, exposing the lettuce crop (red leaf) to the full sun two weeks before harvest. This is akin to the use of movable tunnels to expose the crop to full sun for a short period before harvest to boost the nutritional quality of lettuce. Since the brief exposure of crops to full sun was toward the end of the growing season, it minimizes the possible negative impact typically observed on the biomass accumulation and aesthetic quality of the crop grown in open fields. Because clear poly, not treated with UV inhibitor, may be susceptible to structural breakdown and hence less durable, the use of movable tunnels with the standard poly may be a more promising option for commercial high tunnel crop production. In addition, altering solar radiation using selective poly covers and using shade cloth can also enhance the accumulation of certain essential nutrients such as protein, potassium, magnesium and sulfur in green leaf lettuce. Thus, modulating the solar radiation using photo-selective poly covers in high tunnel production may prove to be an important factor in improving the nutritional quality of lettuce.
Author Contributions: C.B.R. was the principal investigator of the study and was responsible for managing the research activities and writing the manuscript. M.L. conducted field and lab experiments and was involved in data collection and analyses. C.R. was responsible for setting up the field study, high tunnel layout, and crop management. W.W. assisted in the phytochemical analyses. E.P. was involved in field study and crop management and K.G. was involved in field crop management. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by USDA-NIFA, grant number 2016-67017-24712.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: Authors declare no conflict of interest.

References

1. Lamont, W.J. Overview of the use of high tunnels worldwide. HortTechnology 2009, 19, 25–29. [CrossRef]
2. Bruce, A.B.; Maynard, E.T.; Farmer, J.R. Farmers’ perspective on challenges and opportunities associated with using high tunnels for specialty crops. HortTechnology 2019, 29, 290–299. [CrossRef]
3. Carey, E.E.; Jett, L.; Lamont, W.J.; Nennich, T.T.; Orzolek, M.D.; Williams, K.A. Horticultural crop production in high tunnels in the United States: A snapshot. HortTechnology 2009, 19, 37–43. [CrossRef]
4. Knewtson, S.J.B.; Carey, E.E.; Kirkham, M.B. Management practices of growers using high tunnels in the central great plains of the United States. HortTechnology 2010, 20, 639–645. [CrossRef]
5. Beecher, G.R. Nutrient content of tomatoes and tomato products. Exp. Biol. Med. 1998, 218, 98–100. [CrossRef] [PubMed]
6. Kottiková, Z.; Lachman, J.; Hejtmánková, A.; Hejtmánková, K. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT-Food Sci. Technol. 2011, 44, 1703–1710. [CrossRef]
7. Oh, M.M.; Carey, E.E.; Rajashekar, C.B. Antioxidant phytochemicals in lettuce grown in high tunnels and open field. Hortic. Environ. Biotechnol. 2011, 52, 133–139. [CrossRef]
8. Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; Waterland, N.L. Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). J. Food. Compost. Anal. 2016, 49, 19–34. [CrossRef]
9. Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr. Cancer 1992, 18, 1–29. [CrossRef]
10. Prior, R.L.; Cao, G. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. HortScience 2000, 35, 588–592. [CrossRef]
11. Muller, O.; Krawinkel, M. Malnutrition and health in developing countries. Can. Med. Assoc. J. 2005, 173, 279–286. [CrossRef] [PubMed]
12. Tulchinsky, T.H. Micronutrient deficiency conditions: Global health issues. Public Health Rev. 2010, 32, 243–255. [CrossRef]
13. USDA, Food and Nutrition Service, Dietary guidelines for Americans. 2020. Available online: http://www.fns.usda.gov/cnpp/dietaryguidelines-americans (accessed on 1 July 2021). [CrossRef]
14. Heber, D. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad Med. 2004, 50, 145–149.
15. Woolley, A.; Sumpter, S.; Lee, M.; Xu, J.; Barry, S.; Wang, W.; Rajashekar, C.B. Accumulation of mineral nutrients and phytochemicals in lettuce and tomato grown in high tunnel and open field. Am. J. Plant Sci. 2019, 10, 125–138. [CrossRef]
16. Zhao, X.; Iwamoto, T.; Carey, E.E. Antioxidant capacity of leafy vegetables as affected by high tunnel environment, fertilisation and growth stage. J. Sci. Food. Agri. 2007, 87, 2692–2699. [CrossRef]
17. Thoma, F.; Somborn-Schulz, A.; Schlehuber, D.; Keuter, V.; Deerberg, D. Effects of light on secondary metabolites in selected leafy greens: A Rev. Front. Plant Sci. 2020, 11, 1–497. [CrossRef] [PubMed]
18. Paradiso, R.; Proitti, S. Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED Systems. J. Plant Growth Reg. 2021. [CrossRef]
19. Krizek, D.T.; Britz, S.J.; Mirecki, R.M. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. Physiol. Plant. 1998, 103, 1–7. [CrossRef]
20. Hemming, S.; Dueck, T.; Janse, F.; van Noostr, F. The effect of diffuse light on crops. Acta Hort. 2008, 801, 1293–1300. [CrossRef]
21. Lang, K.M.; Nair, A.; Moore, K.J. Cultivar selection and placement of shadecloth on Midwest high tunnels affects colored bell pepper yield, fruit quality, and plant growth. HortScience 2020, 35, 550–559. [CrossRef]
22. Luthria, D.L.; Mukhopadhyay, S.; Krizek, D.T. Content of total phenolics and phenolic acids in tomato (Lycopersicon esculentum Mill.) fruits as influenced by cultivar and solar UV radiation. J. Food. Compost. Anal. 2006, 19, 771–777. [CrossRef]
23. Gude, K.M. Altering solar light with high tunnel coverings to improve nutrition of lettuce and tomato. Ph.D. Thesis, Kansas State University, Manhattan, KS, USA, 2020.
24. Lee, M.; Xu, J.; Wang, W.; Rajashekar, C.B. The effect of supplemental blue, red and farred light on the growth and the nutritional quality of red and green leaf lettuce. *Am. J. Plant Sci.* 2019, 10, 2219–2235. [CrossRef]

25. Gude, K.M.; Rajashekar, C.B.; Cunningham, B.; Kang, Q.; Wang, W.; Lee, M.; Rivard, C.L.; Pliakoni, E.D. Effect of high tunnel coverings on antioxidants of breaker and light red tomatoes at harvest and during ripening. *Agronomy* 2020, 10, 1639. [CrossRef]

26. Nakata, M.; Mitsuda, N.; Herde, M.; Koo, A.J.; Moreno, J.E.; Suzuki, K.; Howe, G.A.; Ohme-Takagi, M. A bHLH-type transcription factor, ABA-inducible bHLH-type transcription factor/JA-associated MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in Arabidopsis. *Plant Cell.* 2013, 25, 1641–1656. [CrossRef]

27. Ainsworth, E.A.; Gillespie, K.M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* 2007, 2, 875–877. [CrossRef] [PubMed]

28. Miller, N.J.; Rice-Evans, C.A. Spectrophotometric determination of antioxidant activity. *Redox Rpt.* 1996, 2, 161–171. [CrossRef]

29. Pennycooke, J.C.; Cox., S.; Stushnoff, C. Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (Petunia x hybrida). *Environ. Exp. Bot.* 2005, 53, 225–232. [CrossRef]

30. Milton, K.; Dintzis, F.R. Nitrogen-to-protein conversion factors for tropical plant samples. *Biotropica* 1981, 13, 177–181. [CrossRef]

31. García-Macías, P.; Ordidge, M.; Vysini, E.; Waroonphan, S.; Battey, N.H.; Gordon, M.H.; Hadley, P.; John, P.; Lovegrove, J.A.; Wagstaffe, A. Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. *J. Agri. Food. Chem.* 2007, 55, 10168–10172. [CrossRef]

32. Lee, M.; Rivard, C.; Pliakoni, E.; Wang, W.; Rajashekar, C.B. Supplemental UV-A and UV-B affect the nutritional quality of lettuce and tomato: Health-promoting phytochemicals and essential nutrients. *Am. J. Plant Sci.* 2021, 12, 104–126. [CrossRef]

33. Hipol, R.L.B.; Dionisio-Sese, M.L. Impact of light variation on the antioxidant properties of red lettuce. *Electron. J. Biol.* 2014, 10, 28–34.