

Article



# Integrated Control of Powdery Mildew Using UV Light Exposure and OMRI-Certified Fungicide for Greenhouse Organic Lettuce Production

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Abstract: Lettuce (Lactuca sativa L.) is a widely cultivated crop due to its short production cycle and high market demand. However, powdery mildew (Golovinomyces cichoracearum) poses a significant threat, reducing yields by up to 30% in various lettuce cultivars. This greenhouse study, conducted at the Rodale Institute in Pennsylvania, evaluated the impacts of pre-transplant UV light exposure and post-planting application of an OMRI-certified fungicide, potassium bicarbonate (MilStop), on powdery mildew infestation, yield, and nutritional quality of lettuce. The treatment included three factors: (a) UV-B (280 to 315 nm) exposure: treated vs. non-treated, (b) UV-C (100 to 280 nm) exposure: treated vs. nontreated, and (c) fungicide application: treated vs. non-treated, arranged in a factorial randomized complete block design with four replications. Lettuce seedlings (Salanova cultivar) were exposed to UV light before transplanting and later treated with MilStop. The results indicated that the combination of UV-B and MilStop significantly reduced powdery mildew infestation, while UV-C alone showed no significant effect. MilStop application enhanced lettuce yield, with treated plots showing a 44.8% increase in harvestable weight over control plots. While mineral and monosaccharide content were unaffected, UV-B exposure significantly increased total amino acid concentrations, including essential and non-essential amino acids. Pearson's correlation analysis revealed a strong negative relationship between powdery mildew severity and harvestable weight, highlighting the importance of disease management. These findings highlight the potential of integrating UV light treatments and fungicide applications as effective, sustainable strategies for managing powdery mildew, improving lettuce yield, and maintaining nutritional quality in regenerative organic systems.

Keywords: lettuce; MilStop; nutritional quality; powdery mildew; regenerative organic

# 1. Introduction

Lettuce (*Lactuca sativa* L.) is a highly favored crop in indoor farming systems due to its rapid growth cycle and substantial market demand. The versatile culinary uses and



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nutritional qualities of this vegetable make it a popular choice in the United States [1]. In 2023, the U.S. Department of Agriculture reported that lettuce accounted for approximately 20% of the sales for vegetable and melon growers, with a production of 44.4 million cwt. The total sales reached USD 4.12 billion, with Romaine lettuce leading the way with over USD 1.5 billion in sales, making it the top-selling variety of lettuce.

However, indoor lettuce cultivation faces challenges, including susceptibility to diseases like powdery mildew caused by the pathogen, *Golovinomyces cichoracearum*. This pathogen has the potential to cause considerable damage to several types of lettuce and other crops, including artichokes, chicory, and their seedlings. This obligate biotrophic fungi captures nutrients from the living host plants. Pathogenesis starts with the pathogen colonizing leaf surfaces and developing conidiophores and conidia throughout the infection court, resembling white powdery patches, as distinctive signs of the pathogen's presence.

The powdery mildew disease limits plant photosynthesis function and displays symptoms that include chlorotic tissues, leaf deformation and necrotic decay, and growth delay with potential plant death [2]. To our knowledge, this pathogen was reported as the dominant disease in lettuce greenhouses in the Pocono Mountains, Pennsylvania, United States, which can cause up to a 30% reduction in yield and a decrease in the marketability of the product. Disease management techniques, including the use of chemicals and biopesticides and decision-based management methods, have been extensively researched on powdery mildew in various regions around the world [3].

Potassium bicarbonate, renowned for its antibacterial properties, has emerged as an environmentally safe alternative to traditional copper- and sulfur-based fungicides, in accordance with the Environmental Protection Agency (EPA) guidelines [4]. Research, including a greenhouse trial at the University of Florida, has shown that potassium bicarbonate, manufactured as MilStop<sup>®</sup>, is far more effective than other bio-fungicides in reducing powdery mildew. While high concentrations of potassium bicarbonate can degrade the quality of fruits and vegetables [5], its controlled application effectively prevents disease progression. Annual management costs for powdery mildew are estimated at USD 370 per acre, with additional economic losses attributed to reduced crop yields caused by this pathogen [6,7].

Ultraviolet (UV) radiation encompasses wavelengths from 100 to 400 nm and is categorized into three bands: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). UV-C radiation is utilized in Ultraviolet Germicidal Irradiation (UVGI) for its potent antimicrobial properties, effectively disinfecting air, water, and surfaces by inactivating microorganisms. Lettuce is a model crop for horticulture fluorescent research because of its adaptability to both the light wavelength and flow density. Exposure to specific UV wavelengths can significantly impact plant growth, nutritional content, and leaf coloration [8,9].

While UV-A and UV-B radiation naturally reach the Earth's surface, UV-C is absorbed by the ozone layer and is absent in natural sunlight at ground level [10]. Research studies have confirmed that applying UV-C radiation at an intensity range of 60 to 170 J m<sup>-2</sup> effectively suppresses powdery mildew without causing harm to the host plants [11]. Exposing plants to UV-B light will also promote the production of defense-related bioactive components. UV-B light exposure induces the synthesis of various amino acids and other metabolites such as flavonoids, phenolic acids, and alkaloids that play critical roles in plant defense mechanisms [12,13]. UV-B exposure has been shown to enhance the production of phenolic compounds and improve antioxidant capacity, contributing to its nutritional value and resistance to pathogens in lettuce plants [14].

Developing innovative approaches to enhance management practices for vegetable growers, particularly within organic agroecosystems, has been a significant area of research for scientists worldwide. Innovations can arise from introducing new technologies or integrating existing methods into production systems. Although the individual applications of UV light and potassium bicarbonate have been studied, their combined use for managing powdery mildew in regenerative organic greenhouses remains underexplored. Therefore, we designed this study to explore the effectiveness of this integrated approach.

Powdery mildew spores commonly infest plants in the germination room where seedlings are produced, and the disease is then transferred to the main growing greenhouses or fields by the seedlings. In the current research, pre-transplanting exposure to UV-C light was used to prevent the transfer of powdery mildew spores by seedlings, restricting their impact and inhibiting their germination on leaves. Additionally, plants were exposed to UV-B light to boost metabolites that increase resistance to pathogens.

Overall, our study combined pre-transplanting UV light exposure with post-planting treatment using an Organic Material Review Institute (OMRI)-certified/listed fungicide. The effects of these factors and their interactions on infestation levels, yield, marketability, and nutritional quality of the produce were evaluated. The goal of this study was to provide organic growers with additional tools and techniques for controlling powdery mildew in a regenerative organic system. We hypothesized that by leveraging the synergistic effects of UV radiation and biocompatible fungicides would enhance plant health and productivity while aligning with organic farming principles.

# 2. Materials and Methods

# 2.1. Study Location

This research was conducted in 2023, in a temperature- and humidity-controlled greenhouse located in the Pocono Mountains, Pennsylvania, United States within USDA cold hardiness zone 6a. A custom-built UV light chamber, equipped with UV-C and UV-B lamps (Eco Pure series, American Ultraviolet Company, Lebabon, IN, USA), was installed in the germination room to expose lettuce seedlings to UV radiation for the duration of the study (Figure 1).



**Figure 1.** Custom-built UV light chamber used for UV-B and UV-C application on the lettuce seedlings in the current study. UV-B and UV-C lamps were installed on the top panel of the wooden structure, and the outer door remained closed during operation.

#### 2.2. Equipment Calibration

UV-C light intensity was measured at various distances from the light source using a UV-C meter. Data collected from 14 distinct distances were used to develop a nonlinear regression model to estimate the energy received by plants exposed to UV-C radiation as a function of distance (Figure 2). According to this model, to expose the tested seedlings to an intensity of 130 to 150 J m<sup>-2</sup>, the seedling trays were placed at a distance of 7 cm from the UV lamp. The duration of exposure to UV-C light and its intensity were selected based on previous research, which reported up to 90% efficacy in restricting the germination of powdery mildew spores [15]. Hence, through this study, we kept this constant distance for both UV-B and UV-C light treatment on lettuce seedlings.



**Figure 2.** Nonlinear model adjusted to represent UV-C light intensity as a function of distances between the source of light and the targets. The determination coefficient ( $R^2$ ) of the fitted model is also shown.

## 2.3. Plant Material

During the winter of 2023, lettuce seeds (Salanova cultivar) were sown in seedling trays (50 seedlings per tray) filled with potting soil supplied by ORGANIC MECHANICS<sup>®</sup>, Kennett Square, PA, USA. Approximately 1500 seeds were sown in January, March, and May. Germination and growth occurred under controlled environmental conditions. At three weeks of age, the seedlings were inoculated with a spore suspension containing  $12 \times 10^4$  powdery mildew spores per mL. A total of 1000 mL of this suspension was evenly distributed over an 80 m<sup>2</sup> cultivation area. After inoculation, the area was monitored to maintain optimal conditions for disease development, ensuring consistent infestation across all seedlings.

## 2.4. Experimental Design

This study ensured a uniform level of powdery mildew infestation across all seedlings to allow the evaluation of the treatment effectiveness. A completely randomized block design was adopted with eight treatments including one untreated control, replicated four times. The treatments consisted of a combination of three factors: (a) UV-B exposure (2 levels): treated vs. non-treated, (b) UV-C exposure (2 levels): treated vs. non-treated, and (c) Fungicide application (2 levels): treated vs. non-treated. Treatment that neither received UV exposure nor treated with fungicide was considered a control. A total of 32 plots (each plot of 3 m<sup>2</sup> size) were established for each experiment, with 40 plants per plot. The entire experiment was repeated three times over the period between January and June 2023.

#### 2.5. Ultraviolet Light Exposure

For UV-B application, seedlings were exposed to UV-B light (280 to 315 nm wavelength) for 10 min at an intensity of 150 J m<sup>-2</sup> (394  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>); for UV-C, seedlings were exposed to UV-C light (100 to 280 nm wavelength) for 5 s at an intensity of 150 J m<sup>-2</sup> (350  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), and for the cases in which seedlings received both UV types, the UV-C was followed by UV-B application, adopting the same intensity and time used of the respective single UV types exposure. Control seedlings were not exposed to any UV light, serving as a baseline to assess the effects of UV light treatments.

#### 2.6. Greenhouse Growth Conditions

After UV light treatments, the seedlings were kept in the germination greenhouse overnight. The following day, all seedlings were transplanted into a greenhouse at Pocono Organics Farm, Pocono Mountain, Pennsylvania. The seedlings were planted in Clymer loam soil with a pH of 7.2, organic matter content of 5.88%, and total nitrogen, phosphorus, and potassium levels of 0.36%, 95.61 mg kg<sup>-1</sup>, and 507.88 mg kg<sup>-1</sup>, respectively. Experimental plots were irrigated using drip tapes to maintain soil moisture at the level of field capacity. Greenhouse temperature and humidity data recorded during this research are presented in Figure 3.



**Figure 3.** Canopy temperature and humidity at different growth stages of lettuce production in a controlled greenhouse. The experiment was repeated three times, with UV light applied before transplantation on each occasion. MilStop was applied twice during each trial, as shown in the production timeline.

#### 2.7. Fungicide Application

Beyond UV-B and UV-C factors, another one involved the use of an OMRI-certified fungicide (potassium bicarbonate, formulated/manufactured as MilStop<sup>®</sup>, by BioWorks, Victor, NY, USA), which had two levels: MilStop treated and non-treated. All non-treated levels, either one main factor or combinations, were considered as control.

MilStop was prepared by mixing 28 g of powder with 9.5 L of distilled water. This solution was applied using a 1-gallon size sprayer and sprayed on the plants twice during the lettuce growing season. The first application occurred seven days after transplantation, with a two-week interval between subsequent applications.

#### 2.8. Disease Evaluation

During the lettuce growing season, a single trained individual performed systematic leaf sampling and plant photography to ensure consistency in data collection. Leaf samples were selected randomly from different canopy levels to capture variability in disease progression. Photographs of sampled plants were taken under uniform lighting conditions to minimize variability in image analysis. The collected photographs were processed manually to estimate the severity of powdery mildew infestation. Disease severity was visually assessed using a standardized rating scale from 0 to 5, where 0 = no visible powdery mildew symptoms, 1 = up to 20% of leaf surface area covered by mildew, 2 = up to 40% of leaf surface area covered by mildew, 3 = up to 60% of leaf surface area covered by mildew, 4 = up to 80% of leaf surface area covered by mildew, and 5 = 100% of leaf surface area covered by mildew. Each plant was evaluated three times throughout the growing season to monitor disease progression. Disease severity was determined by averaging scores from multiple leaves per plant to ensure accuracy and account for within-plant variation. In cases of ambiguous scoring, a second visual assessment was performed to validate the initial rating.

## 2.9. Plant Growth Evaluation and Nutritional Contents

Lettuce plants from each treatment plot were hand-harvested 35 days after transplanting. Fresh yield of lettuce was measured and compared across treatments. Following yield measurement, lettuce leaves were bagged and transported in coolers with ice packs to the Rodale Institute's main campus in Kutztown, Pennsylvania, for processing.

The lettuce heads were washed, cut into 2.5 cm size pieces, and frozen at -20 °C. The samples were then freeze-dried, ground in a grinder, subsampled, and sent to the Agriculture Analytical Laboratory at Pennsylvania State University for mineral nutrient analysis including determination of N, P, K, Mg, Ca, S, Mn, Fe, Cu, Zn, and B contents (testing methods are publicly available at https://agsci.psu.edu/aasl/plant-analysis/methods, accessed on 7 February 2024). A second set of subsamples was sent to the Experiment Station Chem Labs at the University of Missouri-Columbia, Columbia, MO for sugar profile analysis, including quantification of glucose and fructose, vitamin C content, and a complete protein amino acid profile, including essential and non-essential amino acids. Testing methods for these analyses are available at https://aescl.missouri.edu/MethRefs.html (accessed on 7 February 2024).

## 2.10. Statistical Analyses

As previously mentioned, the experiment was conducted three times during the study period, with the data averaged for statistical analysis. Analysis of variance (ANOVA) was employed to evaluate the effects of the main factors UV-B, UV-C, MilStop, and their interactions on powdery mildew infestation levels and lettuce yield using SPSS Statistics Software 30.0.0. Results were reported with mean squares and significance levels for main effects and their interactions.

To visualize the treatment effects on powdery mildew infestation levels, total weight, and harvestable yield, a separate one-way ANOVA was performed, treating each combination of treatments as a single factor. Significant differences among treatments were identified using Duncan's multiple range test as a post hoc analysis. This approach provided a detailed comparison of the treatment combinations' effects on response variables.

Pearson's correlation was conducted, in R software (R-4.4.2 version), to assess relationships between response variables, encompassing total amino acids, essential amino acids, non-essential amino acids, glucose, fructose, vitamin C, harvestable yield, and level of powdery mildew infestation. Correlation coefficients were presented in a matrix format to provide a comprehensive overview of variable interrelationships. Statistical significance was evaluated at p < 0.05 unless otherwise stated.

## 3. Results

#### 3.1. Powdery Mildew Infestation Level and Crop Yield

The severity of powdery mildew infestation was significantly influenced by interactions of UV-B, UV-C, and MilStop (Table 1). Notably, the UV-C treatment alone did not have a significant impact on infestation levels. Although trial replication had a significant main effect on the infestation level, total weight, and fresh leaf weight, no significant interactions with treatments were observed; therefore, these interactions are not presented in Table 1.

**Table 1.** Treatment effects on powdery mildew infestation level, total weight, and fresh leaf weight of lettuce production.

Source of Variation	Infestation Level (MS)	Total Weight (MS)	Fresh Leaf Weight (MS)
Trial	2.4 *	51,500.9 *	46,575.5 *
UV-B	1.0	1136.9 NS	809.6 NS
UV-C	0.5	2870.5 NS	2271.5 NS
MilStop	14.7	4863.4 *	4210.1 *
UV-B × UV-C	0.5	1444.5 NS	1254.8 NS
$UV-B \times MilStop$	1.6	1121.2 NS	890.8 NS
$UV-C \times MilStop$	0.5	1.6 NS	0.1 NS
UV-B × UV-C $\times$ MilStop	1.3 *	95.1 NS	60.2 NS

\* p < 0.05 and NS, not significant. MS stands for mean squares. When the interaction effect was significant, the main factor was not reported.

A one-way ANOVA revealed that all treated plots had significantly lower infestation levels compared to the control plots, which received neither UV light nor MilStop (Figure 4). The average infestation level in control plots was 289% higher than in plots treated with MilStop alone. The lowest infestation level (score = 0.75) was recorded in plots treated with MilStop alone, followed by combinations of MilStop and UV-C (score = 0.78), MilStop and UV-B (score = 0.81), and all three treatments combined (score = 0.94) (Figure 4). These results highlight the effectiveness of MilStop and UV-B treatments in reducing infestation levels, which not only improve yield but also enhance the marketability of lettuce, benefiting growers.

Regarding crop yield, the MilStop application significantly influenced both harvestable and total yields of lettuce (Table 1). Furthermore, the mean total weight of lettuce in plots treated solely with MilStop was 116.4 g per plant (Figure 5a,b). All plots treated with MilStop showed slightly higher total weights compared to control (non-treated) plots.



**Figure 4.** Mean ( $\pm$ SD) powdery mildew infestation levels (scale: 0 to 5) on lettuce leaves across different treatments. Treatments with the same capital letters indicate non-significant differences at the 0.05 significance level.



Figure 5. Mean ( $\pm$ SD) total weight (**a**,**b**) and harvestable yield (**c**,**d**) of lettuce produced among the different treatments. Treatments with the same capital letters indicate non-significant differences at the 0.05 significance level.

A similar trend was also noted for the harvested yield, representing the weight of lettuce leaves (Figure 5c,d). The highest harvestable weight per plant (117.2 g) was recorded in plots treated with both UV-C light and MilStop, marking a 44.8% increase over the control. However, lettuce yields with MilStop alone did not differ significantly from those with the combined effect of UV-C and MilStop treatment. These findings indicate that MilStop effectively reduces powdery mildew infestation and enhances lettuce yield. While UV-B and UV-C treatments did not significantly boost yield, they may contribute to disease suppression when used in conjunction with MilStop.

# 3.2. Nutritional Quality

(a) Minerals: As mentioned earlier, plant tissues were sampled, and mineral content analysis was conducted to determine the concentration of various macro- and micronutrients in the plants. However, the results revealed that there was not any significant effect of applied treatments on the mineral contents of the lettuce leaves (Table 2).

Mineral	Control	MilStop	UV-B	UV-C	UV-B × MilStop	UV-C × MilStop	UV-B × UV-C	UV-B × UV-C × MilStop	Statistics
N (%)	$4.6\pm0.3$	$4.5\pm0.4$	$4.7{\pm}0.3$	$4.6\pm0.4$	$4.7\pm0.4$	$4.7\pm0.2$	$4.7\pm0.4$	$4.6\pm0.2$	(48, 7), F = 0.29, p = 0.96
P (%)	$0.6\pm0.2$	$0.6\pm0.1$	$0.6\pm0.2$	$0.6\pm0.1$	$0.6\pm0.2$	$0.6\pm0.1$	$0.6{\pm}0.2$	$0.6\pm0.2$	(48, 7), F = 0.99, p = 0.46
K (%)	$8.0\pm0.5$	$8.2\pm0.7$	$8.1\pm0.5$	$8.3\pm0.6$	$7.8\pm0.5$	$8.2\pm0.5$	$8.0\pm0.5$	$8.2\pm0.7$	(48, 7), F = 0.40, p = 0.90
Ca (%)	$1.4\pm0.2$	$1.4\pm0.2$	$1.4\pm0.1$	$1.4\pm0.2$	$1.4\pm0.2$	$1.4\pm0.1$	$1.3\pm0.2$	$1.3\pm0.1$	(48,7), F = 0.40, p = 0.90
Mg (%)	$0.3\pm0$	$0.39\pm0$	$0.3\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.3\pm0$	$0.3\pm0$	(48, 7), F = 2.11, p = 0.07
S (%)	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	$0.4\pm0$	(48,7), F = 1.63, p = 0.16
Mn (ppm)	$63.3 \pm 11.1$	$71.3\pm11.2$	$61.3\pm3.4$	$61.5\pm9.0$	$67.8 \pm 13.9$	$66.5\pm2.7$	$60.5\pm8.1$	$64.3\pm6.6$	(48,7), F = 1.25, p = 0.31
Fe (ppm)	$154.8\pm41.6$	$209.8 \pm 89.9$	$89.9 \pm 21.8$	$199.8\pm 67.8$	$178.2\pm50.2$	$182.3\pm49$	$164.2\pm26$	$212.6\pm97.5$	(48,7), F = 0.63, p = 0.73
Cu (ppm)	$10.0\pm2.3$	$9.3\pm1.7$	$10.3\pm2.6$	$9.8\pm2.2$	$9.8\pm1.9$	$9.0\pm1.4$	$9.8{\pm}1.9$	$9.3\pm2.3$	(48, 7), F = 0.97, v = 0.47
B (ppm)	$37.5\pm1.4$	$42.7\pm5.4$	$39.2\pm2.3$	$40.3\pm4.1$	$39.2\pm3.7$	$40.0\pm2.4$	$38.2\pm1.6$	$38.2\pm3.0$	(48,7), F = 1.65, p = 0.16
Na (ppm)	$1786.3\pm383$	$1801.8\pm397$	$1643\pm227$	$1819\pm470$	$1755\pm444$	$1730\pm\!\!243$	$1597\pm331$	$1670 {\pm} 287$	(48,7), F = 0.60, n = 0.75
Zn (ppm)	$54.0\pm4.9$	$53.8\pm3.1$	$55.2\pm5.9$	$54.0\pm 6.0$	$53.8\pm3.9$	$53.0\pm2$	$52.5\pm2.7$	$52.0\pm2.5$	(48,7), F = 0.41, p = 0.89

**Table 2.** Mineral contents (Mean  $\pm$  SD) in lettuce leaves across different treatments.

Note: 'F (df within, df between), F-statistic, p-value', where df\_within represents the degrees of freedom within groups, df\_between represents the degrees of freedom between groups, F-statistic is the calculated F-value, and *p*-value is the probability value.

(b) Primary and Secondary Metabolites: The results showed that any applied treatments did not significantly affect the concentration of the two main monosaccharides, glucose and fructose, nor did they impact the content of the main measured secondary metabolite, vitamin C (Table 3). In contrast, the total content of amino acids in the leaves, including several essential and non-essential amino acids, was significantly affected by UV-B light. However, no significant effects of UV-C light and MilStop on the amino acid contents were observed; surprisingly, a significant interaction between MilStop, UV-B, and UV-C was noted. Figure 6 shows the amount of these metabolites in the leaf samples within the plots of the current study.

Table 3. Impact of different treatments on primary and secondary metabolites of lettuce plant during harvesting.

Source of Variation	Glucose (MS)	Fructose (MS)	Total Amino Acids (MS)	Vitamin C (MS)
UV-B	0 NS	0 NS	3.7 *	98.6 NS
UV-C	310.6 NS	11.6 NS	0.8	10.7 NS
MilStop	185.1 NS	42.8 NS	0	1.30 NS
$UV-B \times UV-C$	61.0 NS	3.1 NS	0.1 NS	14.0 NS
$UV-B \times MilStop$	255.6 NS	430.2 NS	0 NS	4.7 NS
$UV-C \times MilStop$	17.8 NS	9.3 NS	0 NS	17.2 NS
UV-B $\times$ UV-C $\times$ MilStop	0.7 NS	130.3 NS	9.9 **	10.7 NS

\* p < 0.05, \*\* p < 0.01, and NS, not significant. MS stands for mean squares. When the interaction effect was significant, the main factor was not reported.



**Figure 6.** Mean ( $\pm$ SD) content of primary and secondary metabolites across different treatments; glucose, fructose, and amino acids were expressed in w/w%, whereas vitamin C was expressed in mg/100 g. Each metabolite was compared across treatments, with the same capital letters indicate non-significant differences at the 0.05 significance level. NS, not significant.

# 3.3. Relationship Between Response Variables

Pearson's correlation analysis revealed that calcium demonstrated a strong positive correlation with harvestable weight and zinc content (Figure 7). In contrast, glucose, fructose, vitamin C, total amino acids, essential amino acids, tryptophan, histidine, and lysine were all significantly negatively correlated with harvestable weight. As expected, a negative correlation was observed between harvestable weight and fungal infestation level. Conversely, the results showed that a higher level of infestation corresponded to increased content of essential amino acids, tryptophan, histidine, and lysine in the lettuce.



**Figure 7.** Pearson's correlation matrix among the measured variables during the experiment. Blue and red represent positive and negative correlations, respectively, and blank represents non-significant relationship at 0.05 significance level.

# 4. Discussion

Our findings align with those of Van Delm, Melis [16], who demonstrated that a UV-C dosage of approximately 90 J m<sup>-2</sup> effectively suppressed fungal infestation. UV-C controls pathogens by damaging their DNA at a cellular level, inhibiting development and preventing conidia production of conidia. Conversely, Janisiewicz et al. [17] reported that a lower UV-C light intensity, around 12 J m<sup>-2</sup>, was also effective in suppressing powdery mildew. These variations could be attributed to the differences in application length and frequency; our study applied specific treatments only once during the crop's lifecycle. Previous research has shown that the effectiveness of UV-C on fungal infestation can be significantly regulated by a specific dark period immediately following radiation exposure, which may have prevented the light-activated DNA repair mechanism from restoring fungal DNA damage caused by UV exposure [17]. This reduces the amount of electrical energy required for efficient UV therapy while simultaneously reducing the risk of damaging plants and making it commercially feasible [18].

In this study, applying MilStop in combination with UV-B or UV-C reduced powdery mildew infestation rates (Figure 4). MilStop controls fungi by desiccating spores and mycelia and increasing plant tissue pH, creating an environment unfavorable for fungal growth. These results align with other's findings; for instance, Onofre et al. [19] demonstrated that applying a UV-C dose of 170 J m<sup>-2</sup> twice weekly, integrated with standard practices such as pesticide treatments, or a lower dose of 85 J m<sup>-2</sup> applied once per week, led to a more substantial reduction in disease incidence compared to untreated controls. They reported that combining twice-weekly UV-C exposure with fungicide treatment improved disease control due to enhanced suppression of new infections emerging between fungicide applications. This improvement could be attributed to the enhanced suppression of new infections emerging between fungicide applications. However, this approach differs from the method used in the current study, where seedlings were exposed to UV-C only once before transplanting.

While previous studies demonstrated the effectiveness of potassium bicarbonate in disease suppression and increasing vegetable yield [5], the effects of UV lights on plant development are less clear [20]. Some research suggests that UV radiation can boost plant development and leaf expansion [21], while others discover inhibitory effects [22–24]. The current study found that UV-B and UV-C light did not inhibit biomass accumulation (Figure 5).

Although various UV wavelengths can have similar effects on plant growth, morphology, and quality characteristics in lettuce production, it is important to consider differences between types of light-emitting diodes (LEDs), such as photon efficacy ( $\mu$ mol J<sup>-1</sup>), photon flux, and considerations for worker safety and photopic vision. Moreover, UV-C photons, which are highly damaging to living organisms, are fully blocked by the Earth's atmosphere [25]. UV-B photons are likewise harmful, but they can have positive effects such as increased secondary metabolite production [26]. UV-B photons are less destructive than UV-C photons and can stimulate or impede plant growth, depending on the species and environmental factors [20]. Photon flux (mmol s<sup>-1</sup>) per watt (J s<sup>-1</sup>) of input power is a critical performance metric for LEDs in horticulture [25]. UVB (280 to 315 nm) LEDs are less sustainable than C (100 to 280 nm) LEDs because they produce fewer photons per unit of input power, requiring more energy to supply the same photon flux.

Lettuce contains varying concentrations of substances such as glucose, fructose, amino acids, and vitamin C, which influence its nutritional value and flavor. UV exposure, particularly UV-A and UV-B wavelengths, plays a crucial role in modulating both primary and secondary metabolic pathways in lettuce. This modulation occurs through complex signaling mechanisms that activate gene expression, leading to altered levels of various nutrients and bioactive compounds [27]. Environmental factors, particularly short wavelength light, can have a variable effect on the levels of these metabolites; however, there is no consensus on which wavelengths are most effective. In the current study, UV-B radiation had a substantial impact on total amino acid content (Table 3). Interestingly, there were no differences in the efficacy of UV-B, UV-C, and their combination, with or without MilStop, on glucose, fructose, and vitamin C levels, which contradicts previous findings that the addition of UV light increased the soluble sugar and vitamin C content [28,29]. These differences in results could be attributed to several factors, such as cultivar selection, plant age, the timing of UV-A or UV-B light treatments, and fungicide application rate. UV light exposure influences a variety of compounds, causing an overall shift in the chemical content of lettuce [20]. Higher absorption of UV light is commonly associated with increased expression of specific genes regulating these chemicals [30,31].

One limitation of the current study was the decision to combine data from multiple trials rather than presenting results separately for each trial. This approach aimed to account for variables such as day length, temperature, and humidity, which caused differences between the trials in a controlled greenhouse. By incorporating the trial number into the model, this study attempted to provide a comprehensive overview of the trials. However, conducting separate analyses for each trial might better elucidate the interactions between plants, pathogens, and integrated management treatments.

# 5. Conclusions

The findings of this research demonstrate that the integration of UV light treatments with OMRI-certified fungicide (MilStop) effectively reduced powdery mildew infestation in organic lettuce production. The combination of UV-B exposure and MilStop resulted in the lowest disease severity, highlighting their potential synergistic effect in suppressing fungal growth. In contrast, UV-C exposure alone did not significantly reduce infestation levels, suggesting that its efficacy may depend on application timing and interaction with other control methods.

Beyond disease control, MilStop treatment significantly improved lettuce yield and marketability, whereas UV treatments alone did not lead to substantial increases in biomass. The findings suggest that while UV-B and UV-C may play a role in disease suppression, their direct impact on yield is limited. Interestingly, UV-B exposure influenced amino acid accumulation, but no significant changes were observed in mineral content, glucose, fructose, or vitamin C levels.

Overall, these results provide organic growers with an effective integrated disease management strategy that aligns with regenerative organic principles. This research highlights the benefits of UV light in greenhouse disease management while acknowledging its potential risks to human operators. To enhance disease suppression without compromising plant health and yield, future studies should explore optimal UV dosages, application frequencies, and potential interactions with environmental conditions. Further investigation is essential to ensure the safety and practicality of UV light as a sustainable agricultural tool.

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# References

- 1. USDA-NASS. *Vegetables 2019 Summary February 2020;* United State Department of Agriculture, National Agricultural Statistics Service: Washington, DC, USA, 2020; p. 101.
- 2. Lebeda, A.; Mieslerová, B. Taxonomy, distribution and biology of lettuce powdery mildew (*Golovinomyces cichoracearum sensu stricto*). *Plant Pathol.* **2010**, *60*, 400–415. [CrossRef]
- 3. Berrie, A.; Passey, T.; Xu, X. Integrating management of powdery mildew with Botrytis in protected strawberries in the UK. *Crop. Prot.* **2022**, 154, 105902. [CrossRef]
- 4. Mitre, I.; Mitre, V.; Sestras, R.; Pop, A.; Sestras, A. Potassium bicarbonate in preventing and control of apple scab. *Bull. UASVM Hortic.* **2009**, *66*, 186–190.
- Fallir, E.; Grinberg, S.; Ziv, O. Potassium bicarbonate reduces postharvest decay development on bell pepper fruits. *J. Hortic. Sci.* 1997, 72, 35–41. [CrossRef]
- 6. Sosa-Zuniga, V.; Valenzuela, Á.V.; Barba, P.; Cancino, C.E.; Romero-Romero, J.L.; Arce-Johnson, P. Powdery Mildew Resistance Genes in Vines: An Opportunity to Achieve a More Sustainable Viticulture. *Pathogens* **2022**, *11*, 703. [CrossRef] [PubMed]
- Moroney, M. Powdery Mildew Resistance in European, North American, and Hybrid Vitis Species. 2019. Available online: https: //www.extension.iastate.edu/wine/powdery-mildew-resistance-european-north-american-and-hybrid-vitis-species/ (accessed on 15 October 2024).
- Shin, Y.-S.; Lee, M.-J.; Lee, E.-S.; Ahn, J.-H.; Kim, M.-K.; Lee, J.-E.; Do, H.-W.; Cheung, J.-D.; Park, J.-U.; Um, Y.-G.; et al. Effect of Light Emitting Diodes Treatment on Growth and Quality of Lettuce (Lactuca sativa L. 'Oak Leaf'). J. Life Sci. 2014, 24, 148–153.
  [CrossRef]
- 9. Son, K.-H.; Lee, J.-H.; Oh, Y.; Kim, D.; Oh, M.-M.; In, B.-C. Growth and Bioactive Compound Synthesis in Cultivated Lettuce Subject to Light-quality Changes. *HortScience* **2017**, *52*, 584–591. [CrossRef]
- Fahey, D.W. Twenty Questions and Answers About the Ozone Layer: 2006 Update. Scientific Assessment of Ozone Depletion: 2006; World Meteorological Organization: Geneva, Switzerland, 2007.
- 11. Patel, J.S.; Radetsky, L.C.; Nagare, R.; Rea, M.S. Nighttime Application of UV-C to Control Cucumber Powdery Mildew. *Plant Health Prog.* 2020, *21*, 40–46. [CrossRef]
- Heuberger, H.; Praeger, U.; Georgi, M.; Schirrmacher, G.; Grasmann, J.; Schnitzler, W.H. Precision stressing by UV-B radiation to improve quality of spinach under protected cultivation. In Proceedings of the VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition, Kissimmee, FL, USA, 25 November 2004; 659p.
- 13. Zeier, J. New insights into the regulation of plant immunity by amino acid metabolic pathways. *Plant Cell Environ.* **2013**, *36*, 2085–2103. [CrossRef]
- Rajabbeigi, E.; Eichholz, I.; Beesk, N.; Ulrichs, C.; Kroh, L.W.; Rohn, S.; Huyskens-Keil, S. Interaction of drought stress and UV-B radiation–Impact on biomass production and flavonoid metabolism in lettuce (*Lactuca sativa* L.). J. Appl. Bot. Food Qual. 2013, 86, 190–197. [CrossRef]

- 15. Gadoury, D.M.; Sapkota, S.; Cadle-Davidson, L.; Underhill, A.; McCann, T.; Gold, K.M.; Gambhir, N.; Combs, D.B. Effects of nighttime applications of germicidal ultraviolet light upon powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and sour rot of grapevine. *Plant Dis.* **2023**, *107*, 1452–1462. [CrossRef]
- 16. Van Delm, T.; Melis, P.; Stoffels, K.; Baets, W. Control of Powdery Mildew by UV-C Treatments in Commercial Strawberry Production. *Acta Hortic.* **2014**, *1049*, 679–684. [CrossRef]
- 17. Janisiewicz, W.J.; Takeda, F.; Glenn, D.M.; Camp, M.J.; Jurick, W.M. Dark Period Following UV-C Treatment Enhances Killing of *Botrytis cinerea* Conidia and Controls Gray Mold of Strawberries. *Phytopathology* **2016**, *106*, 386–394. [CrossRef] [PubMed]
- 18. Janisiewicz, W.J.; Takeda, F.; Nichols, B.; Glenn, D.M.; Ii, W.M.J.; Camp, M.J. Use of low-dose UV-C irradiation to control powdery mildew caused by *Podosphaera aphanis* on strawberry plants. *Can. J. Plant Pathol.* **2016**, *38*, 430–439. [CrossRef]
- 19. Onofre, R.B.; Gadoury, D.M.; Stensvand, A.; Bierman, A.; Rea, M.S.; Peres, N.A. Use of Ultraviolet Light to Suppress Powdery Mildew in Strawberry Fruit Production Fields. *Plant Dis.* **2021**, *105*, 2402–2409. [CrossRef] [PubMed]
- 20. Verdaguer, D.; Jansen, M.A.; Llorens, L.; Morales, L.O.; Neugart, S. UV-A radiation effects on higher plants: Exploring the known unknown. *Plant Sci.* 2017, 255, 72–81. [CrossRef] [PubMed]
- 21. Chen, Y.; Li, T.; Yang, Q.; Zhang, Y.; Zou, J.; Bian, Z.; Wen, X. UVA Radiation Is Beneficial for Yield and Quality of Indoor Cultivated Lettuce. *Front. Plant Sci.* **2019**, *10*, 1563. [CrossRef]
- 22. Krizek, D.T.; Kramer, G.F.; Upadhyaya, A.; Mirecki, R.M. UV-B response of cucumber seedlings grown under metal halide and high pressure sodium/deluxe lamps. *Physiol. Plant.* **1993**, *88*, 350–358. [CrossRef]
- 23. Ohashi-Kaneko, K.; Takase, M.; Kon, N.; Fujiwara, K.; Kurata, K. Effect of Light Quality on Growth and Vegetable Quality in Leaf Lettuce, Spinach and Komatsuna. *Environ. Control. Biol.* 2007, 45, 189–198. [CrossRef]
- 24. Tsormpatsidis, E.; Henbest, R.G.C.; Davis, F.J.; Battey, N.H.; Hadley, P.; Wagstaffe, A. UV Irradiance As a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environ. Exp. Bot.* **2008**, *63*, 232–239. [CrossRef]
- 25. Kusuma, P.; Pattison, P.M.; Bugbee, B. From physics to fixtures to food: Current and potential LED efficacy. *Hortic. Res.* 2020, 7, 56. [CrossRef] [PubMed]
- 26. Dou, H.; Niu, G.; Gu, M. Pre-harvest UV-B radiation and photosynthetic photon flux density interactively affect plant photosynthesis, growth, and secondary metabolites accumulation in basil (*Ocimum basilicum*) plants. *Agronomy* **2019**, *9*, 434. [CrossRef]
- 27. Sonntag, F.; Liu, H.; Neugart, S. Nutritional and Physiological Effects of Postharvest UV Radiation on Vegetables: A Review. J. Agric. Food Chem. 2023, 71, 9951–9972. [CrossRef] [PubMed]
- 28. Lee, M.; Xu, J.; Wang, W.; Rajashekar, C.B. The Effect of Supplemental Blue, Red and Far-Red Light on the Growth and the Nutritional Quality of Red and Green Leaf Lettuce. *Am. J. Plant Sci.* **2019**, *10*, 2219–2235. [CrossRef]
- 29. Yan, Z.; Wang, C.; Li, Z.; Li, X.; Cheng, F.; Lin, D.; Yang, Y. Supplementary White, UV-A, and Far-Red Radiation Differentially Regulates Growth and Nutritional Qualities of Greenhouse Lettuce. *Plants* **2023**, *12*, 3234. [CrossRef] [PubMed]
- Falcone Ferreyra, M.L.; Rius, S.P.; Casati, P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. Front. Plant Sci. 2012, 3, 222. [CrossRef] [PubMed]
- Naikoo, M.I.; Dar, M.I.; Raghib, F.; Jaleel, H.; Ahmad, B.; Raina, A.; Khan, F.A.; Naushin, F. Role and regulation of plants phenolics in abiotic stress tolerance: An overview. In *Plant Signaling Molecules*; Woodhead Publishing: Sawston, UK, 2019; pp. 157–168.

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