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Application of ultraviolet a led as a disinfectant and morphological effects on indoor grown lettuce

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Lettuce (*Lactuca sativa* 'Parris Island'), commonly grown in vertical farms for sustainable production, has been studied for its response to UVA LED light, but its disinfectant potential remains unexplored. Hence, the proposal intends to cover two important aspects, the development of the plant and its disinfection during cultivation, to obtain plants that are ready for consumption. Three LED light treatments were configured: Mode 1 (WBUVA-P) and Mode 2 (WBUVA-C) used White + Blue + UVA (395 nm), with intermittent and continuous application, respectively. The Control applied White + Blue (WB), denoted as Mode 3. A specific evaluation of different parameters, such as disinfection and identification of bacteria, biomass, chlorophyll content (SPAD units), and leaf area (LA), was conducted in the experiment. The most effective results were obtained with Mode 1 (WBUVA-P), achieving a 99.90% disinfection rate and promoting organic matter accumulation, as shown by increased leaf area, fresh weight, and dry weight. In contrast, Mode 2 (WBUVA-C) reached a 99.00% disinfection rate but did not significantly impact organic matter compared to the Control. These results suggest that UVA-LED radiation can be a valuable tool for food production, enhancing disinfection and organic matter content. However, further studies are needed to explore different intermittent UVA-LED emission durations and test other wavelengths.

Keywords Intermittent light, LED, Plants, Plant-microorganism disinfection, UV-A

Lettuce (*Lactuca sativa* 'Parris Island') one of the most grown vegetables in vertical farming (VF) due to the rising demand for food driven by global population growth of billion people[1–3]. VF are cultivation systems that are isolated from the external environment and consist of multiple vertically stacked layers. These farms use artificial light to promote plant growth[4,5]. Light is the primary factor in vertical farming (VF), as it is essential for photosynthesis, plant growth, and nutritional quality. Light-emitting diodes (LEDs) are particularly effective for plant growth because they offer customizable wavelengths, a longer lifespan, and lower heat emissions[6–8]. The wavelengths used for plant growth in VF include ultraviolet (UV) radiation, as well as visible and infrared light[9,10]. Most studies focus on photosynthetically active radiation (PAR), which ranges from 400 to 700 nm[6,11]. UV radiation is divided into three categories: UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm). Midst these, UVB has been the most extensively studied concerning plant responses[9]. In contrast, UVA, which accounts for 95% of the UV radiation reaching Earth, has received less attention. However, research has shown that UVA radiation can promote the vegetative stage of plant growth[6,9,10,12]. Plant responses to UVA are mediated by blue light photoreceptors, including phototropins, cryptochromes, and zeitlupe proteins. These responses are linked to several aspects of plant morphology, physiology, biochemistry, and photosynthesis[1,6,13]. One of the often-overlooked disadvantages of VF is its impact on disease management owing to pathogens, which can negatively affect both crop quality and yield[14]. Additionally, UVA radiation poses concerns as it can lead to the accumulation of reactive oxygen species (ROS). This accumulation may indirectly damage cells and inactivate pathogens present in food[15–18]. Investigating on the effects of UVA on plants indicates that it can lead to increased leaf area and enhanced biomass production. For instance, studies on Chinese kale exposed to a 380 nm wavelength reported a 58.21% increase in fresh weight and a 65.43% increase in dry weight[19]. Similarly, some studies involving lettuce (*Lactuca sativa* L.) noted increased fresh weight, dry weight, and leaf area[1,11].

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A study tested various UVA intensities (30, 20, and 10 µmol m^{-2} s^{-1}) and found that the optimal intensity for enhancing stem diameter and dry weight was 20 µmol m^{-2} s^{-1}[20]. Different authors have reported that UVA light inhibits leaf area growth. Midst these studies, no significant changes in leaf area and dry weight were observed at 400 nm during a 4 day treatment with blue light plus UVA[21]. In another study, a decrease in fresh weight was reported, while the length and width of lettuce, as well as dry weight, showed no significant differences[22]. Finally, exposure to UVC (254 nm) and UVA (365 nm) resulted in a 69% decrease in the germination percentage of a bulb strain[23]. Studies on disinfection using UVA light have shown notable results. For instance, employing UVA with a single and fractionated exposure at 365 nm and an intensity of 183 mW cm^{-2} achieved reductions of log − 3 and log − 3.6[18]. The use of UVA wavelengths (366 and 400 nm) combined with UVC for wastewater disinfection led to a loss reduction of − 4, with no photoreactivation observed[16]. For nutrient solution disinfection, UVA achieved a maximum rate of 94% under optimal conditions[24]. Additionally, there was a 99.7%, 98.7%, and 92.3% disinfection of *S. aureus*, *E. faecalis*, and *E. coli*, respectively[15]. Research has primarily concentrated on the impact of Photosynthetically Active Radiation (PAR) on plants, while the effects of UVA radiation—especially about disinfection—remain largely unaddressed. Most existing literature has focused on disinfection methods for nutrient solutions, creating a gap in understanding the direct application of UVA-LED radiation in indoor crops. Using UVA-LED radiation in these settings could enhance food safety by reducing the bacterial load in lettuce (*Lactuca sativa*) and improving its nutritional quality. This study aims to evaluate the effects of UVA-LED radiation during the vegetative stage of lettuce growth, specifically assessing its efficacy as a disinfectant, its impact on vegetative development, and the nutritional quality of the plants. Additionally, the research compares the effects of continuous versus intermittent radiation emission. The findings of this study will provide a foundation for future research focused on improving wavelengths and exposure times, ultimately contributing to the development of more effective strategies for crop production in controlled environments.

Results

The results with UVA-LED irradiation presented positive effects on certain parameters such as lettuce morphology. Additionally, the UVA-LED emission obtained a disinfection of − 3 Log. This proposal not only provides fundamental evidence that UVA-LED radiation can be implemented in indoor crops from the early vegetative stages without affecting crop yield but also assures its practicality and feasibility from a sanitary point of view (food safety).

Bacterial identification

Several bacteria, such as *E. coli, S. aureus, K. pneumoniae* and *Salmonella* spp., were isolated and identified in the lettuce samples established as control (without radiation). Figure 1A illustrates the results of the selective media with this procedure, showed as indicated in Table 3 in the identification section. The presence of the bacteria's mentioned above were detected by biochemical tests. Positive results for hydrogen sulfide are evidenced by the black coloration generated by the hydrogen sulfide and Klingler tests for *Salmonella* spp., with red color at the top and yellow at the bottom. The positive results for *E. coli* according to indole with a red halo on the surface, citrate with the color change to green, and Kligler Yellow color at the top and bottom, as shown in Fig. 1B. Finally, the presence of *S. aureus* was confirmed in the catalase and coagulase tests, which further validates the results.

At the end of the experiment (30 days after the radiation treatment), in the WB treatment, *E. coli*, a bacterium of sanitary importance, was isolated and identified. On the other hand, with the WBUVA-P and WBUVA-C LED treatments, only *K. pneumoniae* was identified.

Bacterial quantification

The initial bacterial loads of aerobic mesophiles, total coliforms, and yeast fungi in the lettuce samples were 4.14, 3.22, and 3.88 Log, respectively. The evaluation of log inactivation after 15 days of UVA-LED light exposure (WBUVA-P and WBUVA-C) was markedly different from the WB treatment. As shown in Fig. 2, the reduction in aerobic mesophiles was − 2.55 Log for treatment WBUVA-P, − 2.17 Log for WBUVA-C, and − 1.38 Log for the WB treatment. For total coliforms, the log reduction was − 3.0 Log for WBUVA-P, − 1.5 Log for WBUVA-C, and − 1.12 Log for WB. Finally, the disinfection of yeast fungi resulted in − 1.56 Log for WBUVA-P, − 1.18 Log for WBUVA-C, and − 0.905 Log for WB.

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The evaluation of the UVA-LED effect at 15 and 30 days of different treatments revealed significant differences ($p < 0.05$) in the disinfection percentages for aerobic mesophiles and fungi-yeasts when compared to the WB treatment. Specifically, the WBUVA-P treatment showed a significant difference compared to the WBUVA-C and WB treatments after 15 days of radiation.

The disinfection percentages for aerobic mesophiles ranged from 99.65 to 99.66%, while approximately 99.90% was achieved for total coliforms. For fungi-yeasts, the disinfection percentages ranged between 96.87 and 97.28% for the WBUVA-C and WB treatments, showing lower disinfection rates after both 15 and 30 days of radiation.

Table 1 displays the final bacterial loads for each light treatment. The treatments using UVA-LED resulted in the lowest bacterial loads. The WBUVA-P treatment demonstrated a reduction in bacterial load compared to

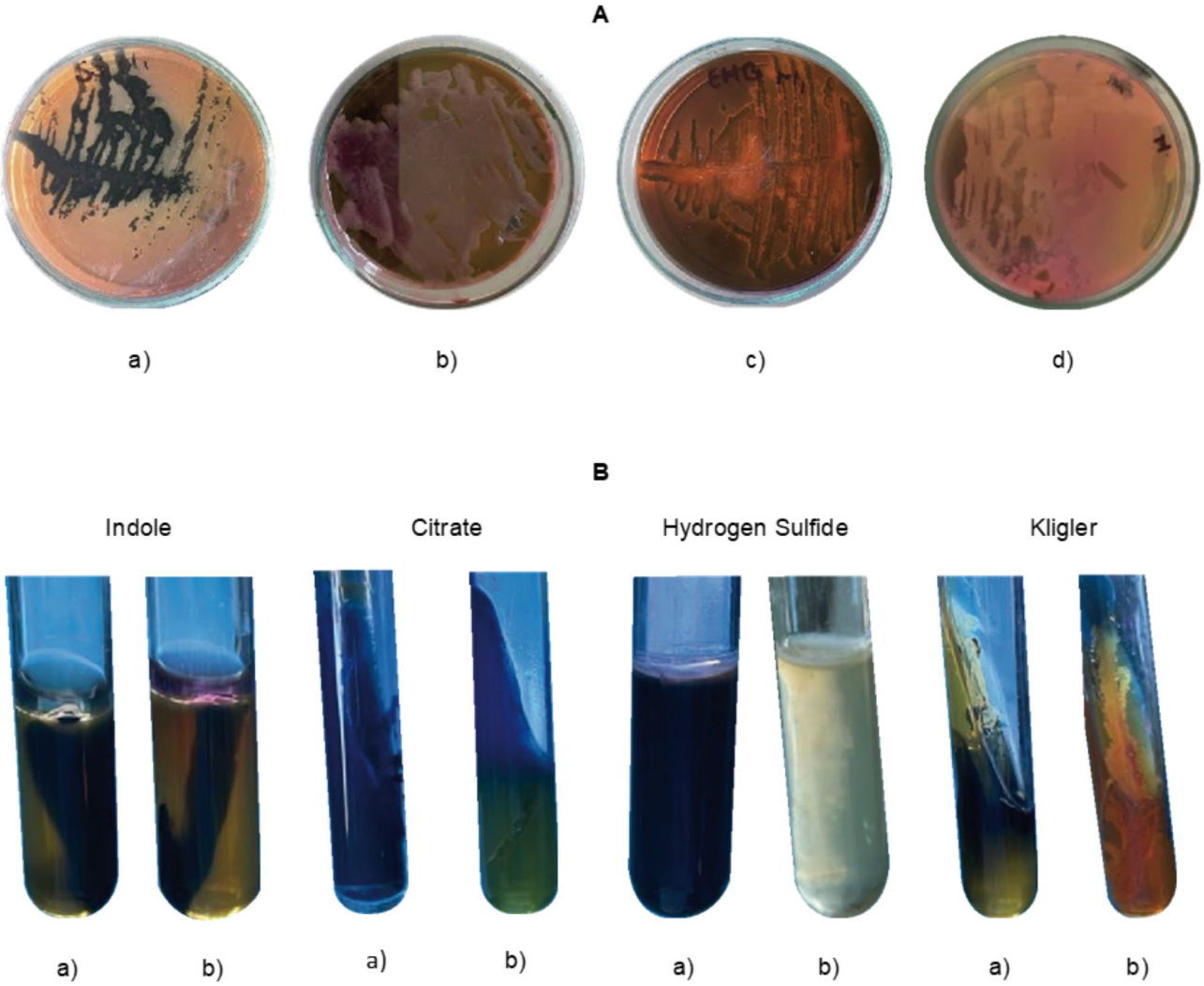

Fig. 1. Bacterial identification. (**A**) Results obtained from the selective media SS, EMB and MSA of the sample without radiation. (**a**) Growth of *Salmonella* spp. in SS. (**b**) Growth of *K. pneumoniae* in EMB. (**c**) Growth of *E. coli* in EMB. (**d**) Growth of *S. aureus* in MSA. (**B**) Biochemical test (**a**). *Salmonella* spp. (**b**) *E. coli*. The tests included Indolee, Citrate, Hydrogen Sulfide and Klingler.

both the WB and WBUVA-C treatments after 15 days. By the 30-day mark, the WBUVA-P treatment maintained its bacterial load, showing no significant increase after the initial reduction. This pattern was consistent across aerobic mesophiles, total coliforms, and fungi/yeast.

An important fact is that in the WB treatment, the final bacterial concentration increased, nearly returning to its initial level, since it falls within the visible spectrum and is not technically a disinfectant.

Morphological analysis

The effect of different treatments on leaf area, fresh weight, dry weight and chlorophyll is shown in Fig. 3. The WBUVA-P treatment stands out, showing a significant increase in leaf area compared to the WBUVA-C and WB treatments. The highest value, approximately 1340, obtained in the WBUVA-P treatment far exceeds the results of the WBUVA-C and WB treatments, which yielded 1100 and 1160, respectively. The leaf area obtained between the UVA-LED treatments presents a panorama with both positive and negative effects due to morphological determinations. Continuous radiation tends to decrease leaf area, while the emission of intermittent radiation leads to a significant increase. In relation to fresh weight, indicating that the WBUVA-P treatment displays significant differences through WBUVA-C and WB. A fresh weight of 100.7 g was obtained for the WBUVA-P treatment, 79 g for WBUVA-C, and 88 g for WB. Furthermore, the WBUVA-P treatment's leaves were not only more robust but also more pigmented, enhancing their visual appeal, showed in Fig. 4. And for DW, shown a substantial increase of (10.4 g) in the WBUVA-P treatment. In contrast, the WBUVA-C and WB treatments recorded values of 8.65 g and 8.46 g, respectively. This underscores the potential of UV-A LED to enhance lettuce biomass and its physiological aspects, as evidenced by the increase in dry weight.

The WBUVA-P treatment showed an increased chlorophyll content compared to the WBUVA-C and WB treatments. Although there was a slight decrease in chlorophyll content in the WB treatment, which was linked

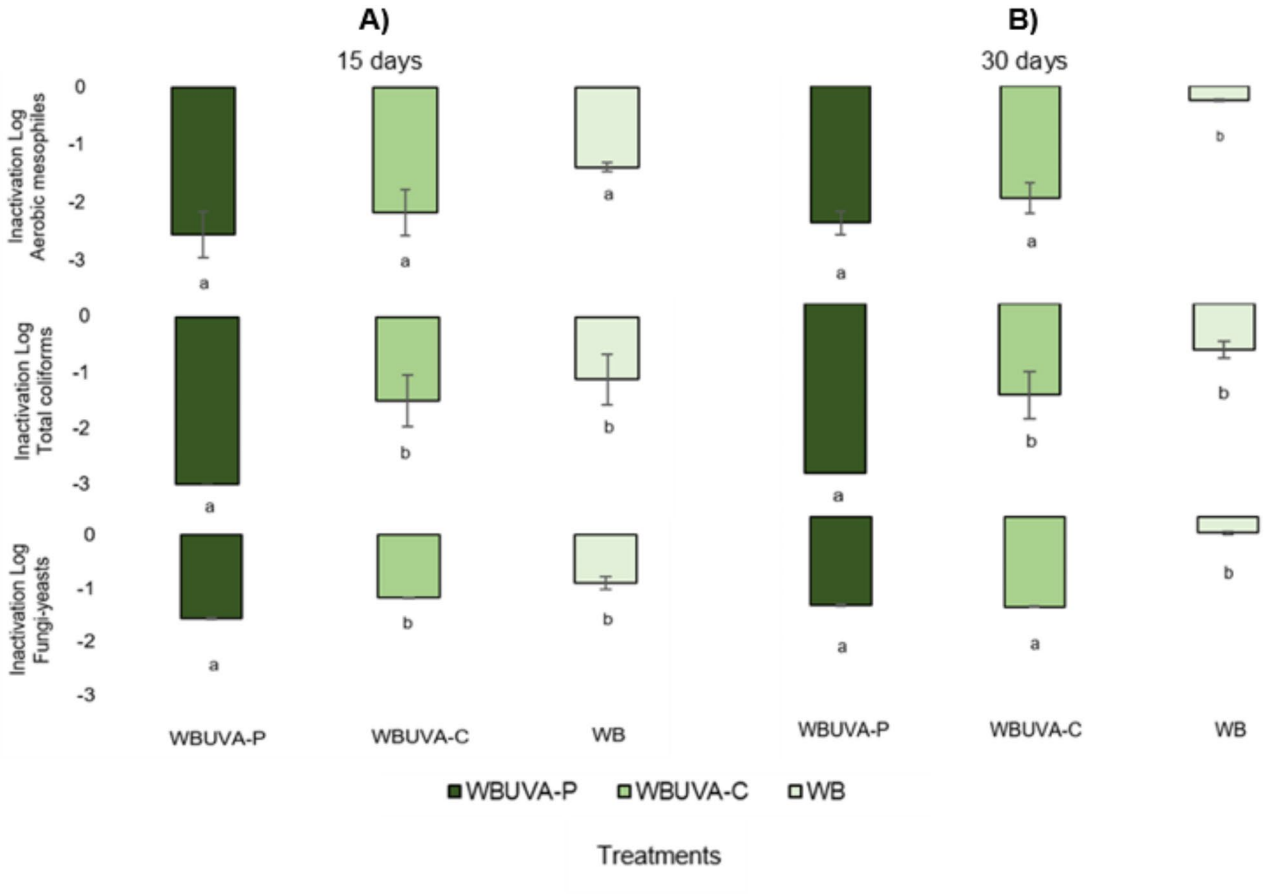

Fig. 2. Bacterial quantification Logarithmic inactivation at 15 and 30 days of irradiation with WBUVA-P, WBUVA-C, and WB treatments for aerobic mesophilic microorganisms, total coliforms, and fungi yeast. Standard deviation (SD) is represented by the error bars, and the significant difference is denoted in alphabetical order (highest to lowest).

Microorganism	Light cycle (days)	Initial load (Log N/No)	Final load (Log N/No)		
			WBUVA-P	WBUVA-C	WB
Aerobic mesophiles	15	4.14	1.62	2.00	2.74
	30		1.64	2.10	3.87
Total coliforms	15	3.22	0	1.98	2.34
	30		0	1.82	2.31
Fungi-yeasts	15	3.88	2.32	2.69	2.98
	30		2.37	2.31	3.58

Table 1. Initial load, final load and disinfection percentage obtained in the experimentation for WBUVA-P, WBUVA-C and WB.

to an environmental factor within the vertical farm, this change was not statistically significant. Notably, the positive effect of the WBUVA-P treatment on chlorophyll accumulation suggests an enhancement in photosynthetic efficiency in plants. The results indicate that UVA-LED exposure has the potential to increase nitrogen levels in plants. Previous studies have established a significant relationship between chlorophyll content and nitrogen availability. This finding implies that UVA-LED treatment not only promotes chlorophyll synthesis but may also enhance nitrogen uptake or utilization in plants. As a result, this synergy could lead to improved photosynthetic efficiency and overall plant health, highlighting the importance of optimizing light treatments in agricultural practices.

Figure 4 shows the morphology of *Lactuca sativa* 30 days after exposure to different treatments: WBUVA-P, WBUVA-C, and WB. Notable differences can be seen among the treatments, especially regarding leaf structure, size, and overall plant development. The plants treated with WBUVA-P and WBUVA-C display distinct

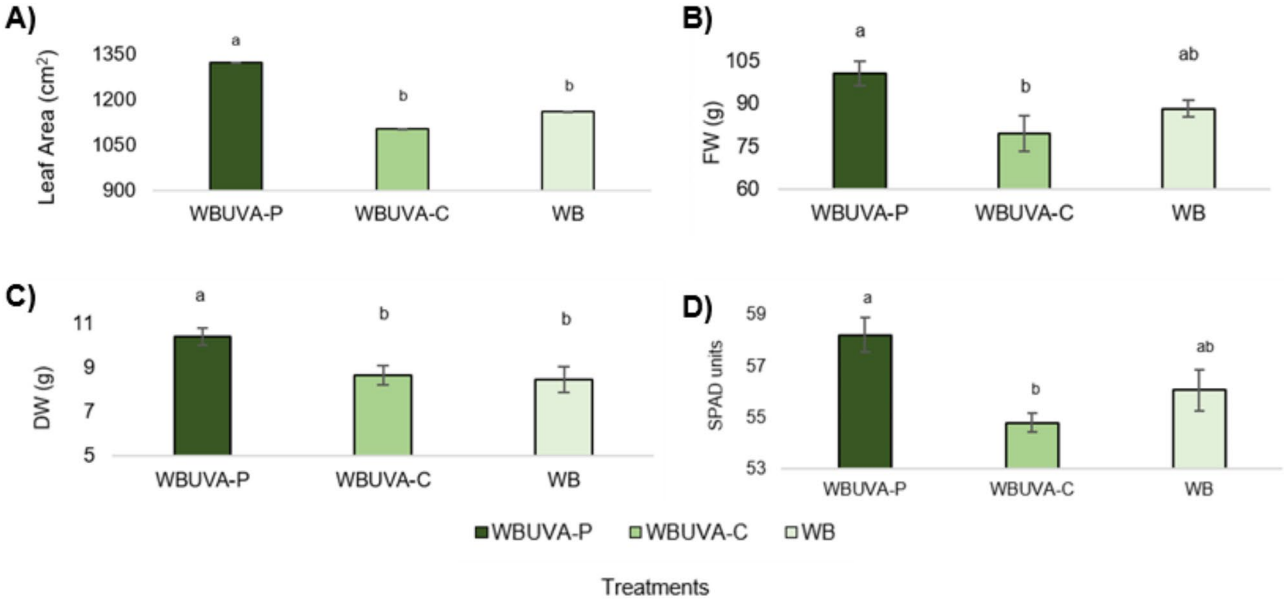

Fig. 3. Illustrates the biomass measurements and chlorophyll content (measured as SPAD) for the various artificial radiation treatments: WBUVA-P, WBUVA-C, and WB. The measurements include (**A**) Leaf Area (LA), (**B**) Fresh Weight (FW), (**C**) Dry Weight (DW), and (**D**) Chlorophyll The standard deviation (SD) is indicated by error bars, and significant differences are noted in alphabetical order from highest to lowest.

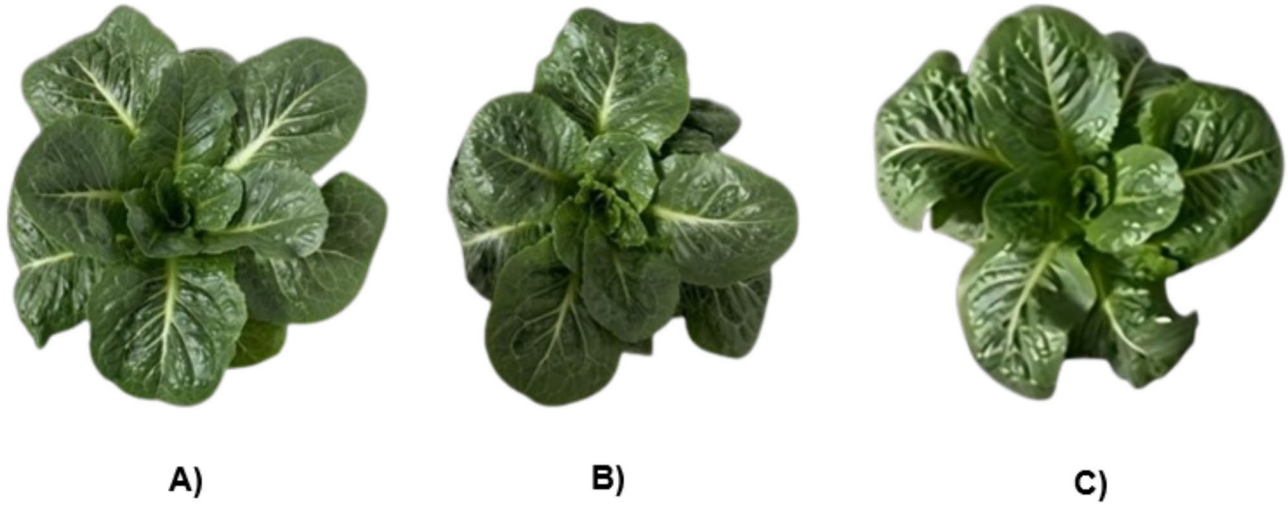

Fig. 4. Lettuce (*Lactuca sativa*) morphology at 30 days after treatments: (**A**) WBUVA-P, (**B**) WBUVA-C, and (**C**) WB.

morphological adaptations when compared to the WB treatment, indicating that UVA exposure affects growth patterns.

Energy consumption

According to the results, treatments adding UVA-LED radiation showed a significant increase in power consumption compared to the WB treatment, with a 155.82, 159.6, and 80.1 KWh, in the WBUVA-P, WBUVA-C, and WB treatments, respectively. Specifically, the integration of UVA radiation led to a 50% more power consumption relative to lighting treatments that do not include UVA. This increase in electrical consumption can be attributed to the additional energy required to power the UVA emitters, which, while enhancing plant growth and chlorophyll production, also places additional energy demands on the system.

Discussion

Our research revealed that the WBUVA-P and WBUVA-C treatments exhibited a higher disinfection capacity compared to the WB treatment. When using radiation at 395 nm, we achieved disinfection rates of 99.0% and 95.87% for total coliforms. Unlike previous studies that focused solely on the disinfection of bacteria in laboratory settings or the response of the plants, this research examined both aspects by evaluating plant response and disinfection during the vegetative stage of the crops. Previous studies have reported bacteria inactivation of 98.7% for *E. coli* and 92.3% for *E. faecalis* when exposed to UVA-LED radiation at 365 nm for 8 h[15]. Similarly, combining TiO_2 with UVA light (in the 365–390 nm range) has shown the potential to achieve an inactivation of up to 4 log in water samples contaminated with *E. coli*[25]. However, these methods are limited to direct applications of radiation on bacteria in Petri dishes, without taking into account the effects on plant growth and the microbial processes involved in culture growth. This research applied longer wavelengths and an exposure time of 16 h, in contrast to other studies that employed shorter wavelengths and exposure times[18]. We achieved superior results in the intermittent disinfection of lettuce samples using WBUVA-P. The effectiveness of the WBUVA-C treatment observed at 30 days suggests that photoreactivation may have occurred. In contrast, WBUVA-P exhibited stable disinfection effects at both 15 and 30 days, indicating the absence of photoreactivation in that treatment. UV radiation disinfects by damaging microbial DNA, primarily through the formation of pyrimidine dimers, which disrupt cell replication and transcription[26]. The results regarding plant response indicated an increase of 13.8% in leaf area, 14.4% in fresh weight, and 20.2% in dry weight for the WBUVA-P treatment compared to the control sample. Additionally, an increase in leaf pigmentation was observed under the WBUVA-P treatment. However, previous studies have primarily focused on plant responses only at the postharvest or germination stages, neglecting the effects of radiation during the growth and development cycle[1,11,27]. The use of UVA radiation in both continuous and intermittent applications is a novel approach compared to previous studies, showcasing its effectiveness in disinfection and promoting plant growth. However, the current literature does not measure the energy consumption of these systems, which is crucial for assessing their viability in agro-industrial settings. Therefore, future research should investigate the combination of different wavelengths and exposure time configurations to find an appropriate balance between microbiological safety and plant development. From an ecological and productive approach, the findings indicated that UVA radiation may represent an alternative strategy to chemical disinfectants, reducing the dependence on synthetic antimicrobial agents and their environmental impact. However, it is still necessary to explore the specific cellular mechanisms by which UVA radiation interacts with plant biological systems and its effect on the beneficial microbiota associated with the crop. While the initial energy cost of implementing UVA-LEDs may be high, their potential to improve the microbiological safety of crops and promote plant growth offers considerable benefits for agricultural production. This enhancement not only ensures the quality of products but also helps reduce the need for chemical input. Additionally, it fosters trust between consumers and producers, paving the way for a more sustainable and technologically advanced agricultural model.

Conclusion

The findings on the effect of multispectral LED light on the cultivation of lettuce (*Lactuca sativa* 'Parris Island') in vertical farming emphasize a sustainable and efficient method for enhancing crop production. The use of UVA LED treatments resulted in increased yields while also improving the nutritional quality of the plants and boosting their defense mechanisms. In this study, UVA radiation (395 nm) positively influenced lettuce growth and development, leading to higher biomass production while helping to maintain the plants' natural defenses and reducing their vulnerability to diseases and pests. This reduction in pathogen load can lessen the dependence on chemical pesticides, thereby promoting more sustainable agricultural practices. Overall, the experiment demonstrated an increase in lettuce growth, development, and disinfection during the vegetative stage when subjected to UVA-LED treatments (WBUVA-P and WBUVA-C). As future work will analyze the relationship between energy consumption and biomass or nutrient production. Additionally, we suggest exploring strategies to improve energy efficiency, such as adjusting exposure parameters, implementing intermittent photoperiods, and utilizing spectral combinations that require less energy.

Methods

Experimental configuration

Figure 5 explains the general configuration of the experiment. The seedlings were grown in a greenhouse located at Invernaderos Zapata in Aguascalientes, Mexico, with geographic coordinates of 22° 14′ 19.0" N and 102° 00′ 44.9" W. They were exposed to solar radiation with temperatures ranging from 28 °C during the day to 14 °C at night for a duration of 21 days. The next step in our study involves randomly selecting seedlings for transplanting and subjecting them to different LED light treatments.

Mode 1 (WBUVA-P): This treatment includes White, Blue, and UVA light with intermittent radiation, where the ultraviolet lamp operates in cycles of 4 h on and 2 h off (4 cycles total). 2). Mode 2 (WBUVA-C): The treatment also consists of White, Blue, and UVA light, but the on/off time aligns with the photoperiod of 16 h on and 8 h off. Control (WB): This mode features only White and Blue light (denoted as Mode 3), with the on/off time matching the photoperiod of 16 h on and 8 h off. After these treatments, the next step will involve measuring the growth and physiological responses of the seedlings to determine the effectiveness of each lighting treatment.

Section "Results" focuses on the bacteriological evaluation, that one is divided into two parts. First, the initial bacterial load present in the sample was identified. Next, a general quantification of the bacteria was conducted, allowing for the evaluation of the food's microbial quality. The first aspect involves bacterial identification conducted on days 0 and 30 of the growth cycle after transplanting. The second aspect focuses on bacterial quantification performed on days 0, 15, and 30 of the growth cycle under the light treatments, where

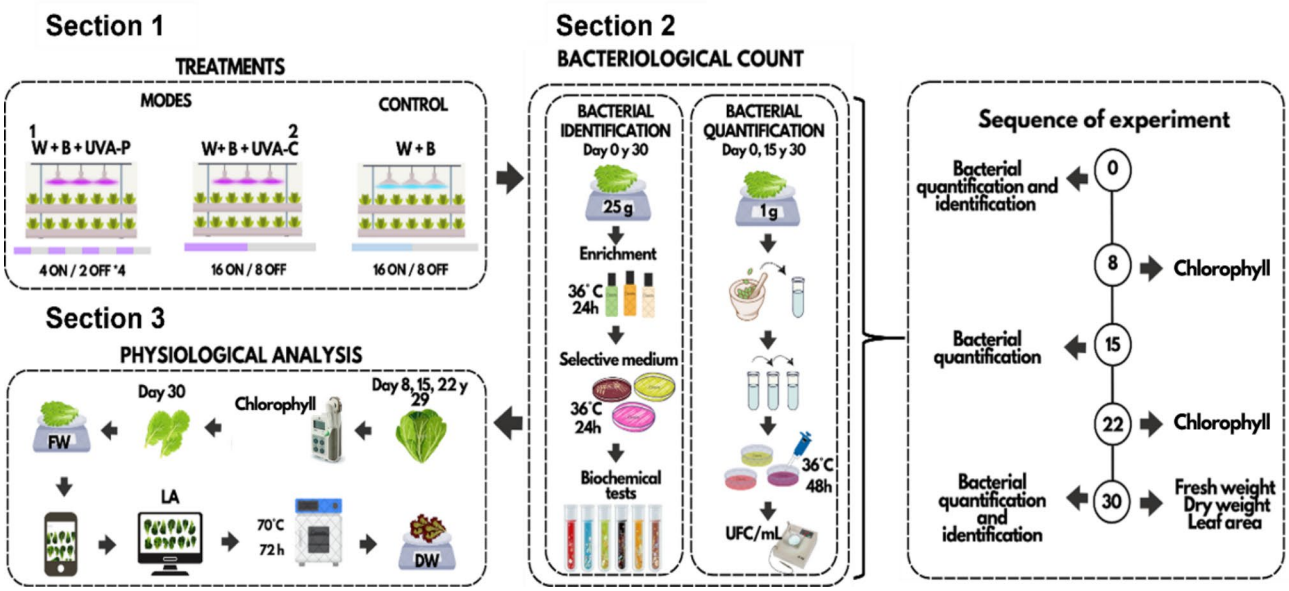

Fig. 5. General scheme of the experiment for growing lettuce plants (*Lactuca sativa* 'Parris Island').

the effectiveness of the disinfectant was evaluated (according to the experiment sequence). Finally, Section "Discussion" focuses on the physiological evaluation of the plants, including the calculation of chlorophyll content. This measurement was conducted weekly to gauge the plants' responses to different LED lighting treatments. The growth measurements were taken concurrently with the lettuce growth and development cycle. At the end of the growth cycle under LED light (day 30), four plants from each treatment were randomly selected to collect data on fresh weight, dry weight, and leaf area (FW, DW, and LA, respectively) through destructive measurement. All parameters were measured in triplicate, and the experiment was replicated twice.

Light treatments

The lamps used in the experiment included RAISETEC cold lamps rated at 18 W, with 18 lamps installed per level. Additionally, TIANLAI LED lamps emitted light at 457 nm, also rated at 18 W, with nine lamps per level. Ultraviolet A radiation was supplied by 24 SAGLITE lamps rated at 36 W, emitting at 395 nm. A timer controlled when the treatments were activated. To characterize the treatments, a quantum sensor (Lightscout Spectrum® Technologies, Inc.) and a USB650 Red Tide spectrophotometer (Ocean Optics, Orlando, FL, USA) were used, with a range from 350 to 1000 nm. Figure 6 shows the emitted wavelengths for the irradiation treatments: (1) WBUVA-P, (2) WBUVA-C, and (3) WB. The characterization of the treatments was carried out using a quantum sensor (Lightscout Spectrum® Technologies, Inc.) and a USB650 Red Tide spectrophotometer (Ocean Optics, Orlando, FL, USA), which has a range from 350 to 1000 nm. Figure 5 illustrates the emitted wavelengths for the irradiation treatments: (1) WBUVA-P, (2) WBUVA-C, and (3) WB.

An important factor in the growth cycle of plants is the photoperiod, which can trigger specific plant phenomena. In this experiment, adjustments were made to the daily duration of light exposure for the crops. Figure 7 shows the cycles of treatment on and off. The Mode 1 WBUVA-P treatment was set with a 16-h on cycle (applying both White and Blue light quality) and included four UVA-P cycles, consisting of four hours of light followed by two hours off, thereby completing its 16-h emission period[28–31]. The Mode 2 (WBUVA-C) involves a cycle of 16 h on and 8 h off, employing all light qualities. In contrast, the Mode 3 (WB) maintains a similar 16-h on and 8-h off cycle, but it is specifically for the White and Blue wavelengths. The intensity of the White and Blue light is set at 230 µmol m^2 s^{-1} (comprising 184 White and 46 Blue), while the UVA light has an intensity of 19 µmol m^2 s^{-1}. This research has the potential to inspire new approaches in plant growth experiments.

Growth conditions

Lettuce seedlings were placed in 450 ml Styrofoam cups filled with a substrate composed of 90% peat moss and 10% perlite. The seedlings were arranged on racks in a controlled chamber, each accommodating 48 plants. The layout of each rack measured 49 cm in length, 51 cm in width, and allowed for 9 cm of spacing between each lettuce plant. The environmental conditions established during the experiment were a temperature of 20 ± 2 °C and 60–80% relative humidity within the LED lighting treatment. Irrigation was carried out with "Steiner" nutrient solution at an osmotic potential of 0.547 Mpa, electrical conductivity (EC) of 1.5 dS m^{-1}, pH 5.5. During the experiment, the environmental conditions were maintained at a temperature of 20 ± 2 °C and a relative humidity of 60% to 80% under LED lighting. Irrigation was provided using a "Steiner" nutrient solution with an osmotic potential of 0.547 MPa, an electrical conductivity (EC) of 1.5 dS m^{-1}, and a pH of 5.5. The nutrient solution was meticulously prepared with a precise concentration of macronutrients NO^{3-}, H_2PO^{4-}, SO_4^{2-}, K^+, Ca^{2+}, and $Mg^{2+}(meL^{-1})$: 12.0, 0.8, 6.8, 5.7, 9.0 y 4.0, respectively. Micronutrients: $FeSO_3 7H_2O$, $MnSO_4 4H_2O$,

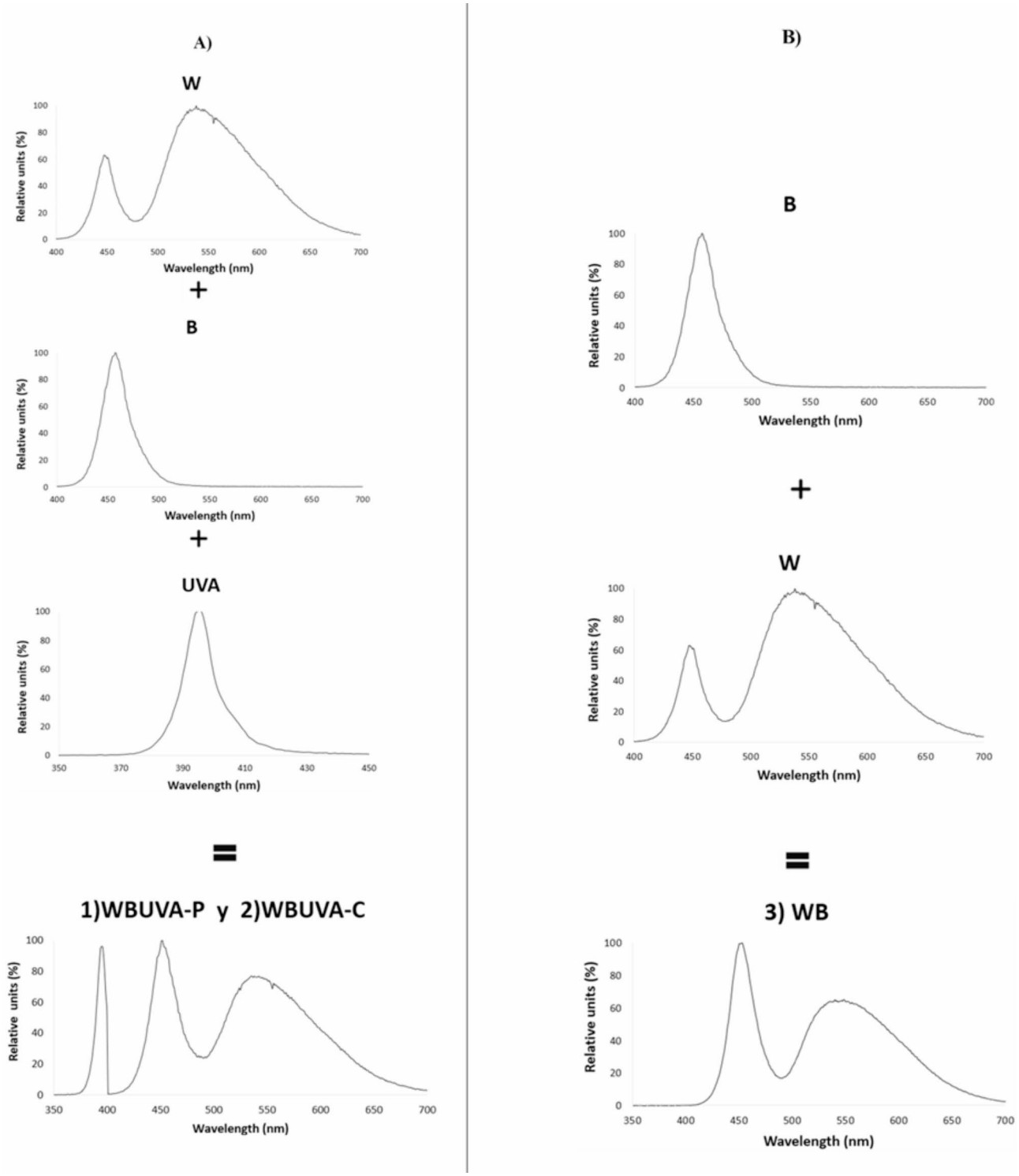

Fig. 6. Configuration of light recipes. (**A**) Individual spectra for White, Blue, UV-A, and the full spectrum treatments WBUVA-P and WBVA-C. (**B**) White and Blue spectra for the full spectrum WB treatment.

$CuSO_4 5H_2O$, $ZnSO_4 7H_2O$, $Na_2[B_4O_5(OH)_4]\ 8H_2O$, and $Na_2MoO_4 2H_2O$ had a concentration (me L^{-1}) of 19.9, 2.22, 0.388, 1.44, 2.46, and 0.12, correspondingly.

Bacterial identification

Microorganisms were isolated and identified from lettuce samples collected from four randomly selected heads of lettuce. The bacteriological analysis consisted of two stages: identification and quantification. Bacterial identification focuses on species such as *E. coli, Salmonella* spp., and *Staphylococcus aureus*. For bacterial

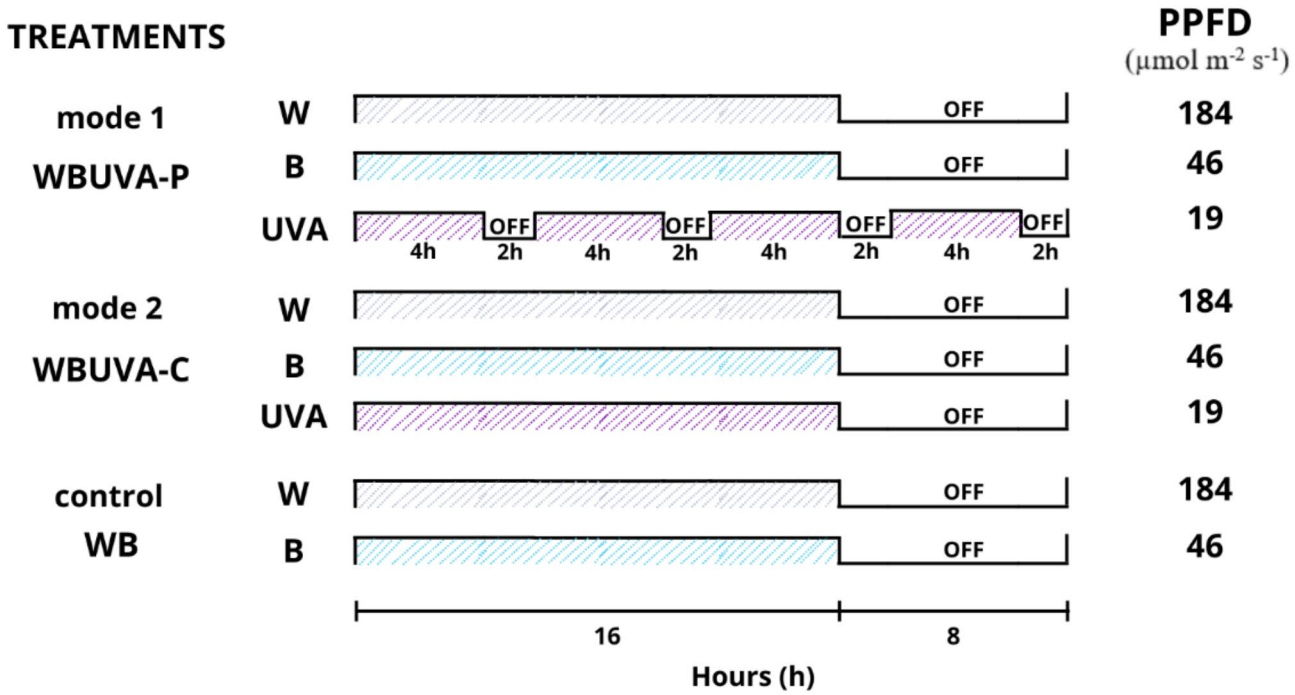

Fig. 7. The photoperiod distribution for light treatments (WBUVA-P, WBUVA-C, and WB) throughout the development and growth cycle of lettuce.

Determination	Medium	Interpretation	Microorganism	Broth
Quantification (UFC/mL)	VRBL (Violet Red Bile Agar)	N/A	Total coliforms	N/A
	SMA (Standard Methods Agar)		Aerobic mesophiles	
	PDA (Potato Dextrose Agar)		Fungi and yeasts	
Identification	EMB (Eosin-methylene blue)	Darck to black colonies with a metallic green sheen	*E.coli*	BGBB (Brilliant Green Bile Broth)
	SS (*Salmonella* spp. and shigella)	Beige or black colonies	*Salmonella* spp.	TT (Tetrathionate Broth Base)
	MSA (Mannitol Salt Agar)	Yellow colonies and change of the medium to yellow	*S. aureus*	BHI (Brain Heart Infusion Broth)

Table 2. Agars and broths are utilized for the quantification and identification of bacteria.

quantification, the reliable pour plate technique was employed, allowing for the precise evaluation of aerobic mesophiles, total coliforms, and yeasts (Table 2). This process is essential for assessing the sanitary quality of the crops and ensuring their safety for human consumption.

The initial seedling sample load was established prior to subjecting the plants to light treatment. The experimental groups were analyzed 15 and 30 days after exposure to radiation. Each sample consisted of a random selection of four lettuce plants per treatment. For all experimental conditions, tests were conducted in triplicate, resulting in a sample density of 12 lettuce plants per treatment.

The bacterial identification process involved enriching the sample in selective broths. We used BGBB (BIOXON) for Escherichia coli, TT (MCDLAB) for *Salmonella* spp., and BHI (BIOXON) for Staphylococcus aureus. The analysis process began by mixing 25 g of the sample with 12 mL of the respective selective broth. After incubating the broth, we performed a second culture using a nichrome loop. Table 3 includes the selected media used, the bacteria tested in this experiment, and the conditions of the biochemical tests. These tests were applied to the colonies grown on the selective media, which included EMB, SS, and MSA. The tests were conducted at 0 and 30 days into the growth cycle (see Fig. 5 for the experimental sequence). Overall, the methodology used in this process was based on and modified from the protocols reported by Meteab et al.[32], Shah et al.[33], Pumipuntu et al.[34], and Ramchandar[35].

Bacterial quantification

Bacterial quantification was conducted using the pour plate technique with specific culture media: VRBL (BIOXON) for total coliforms, SMA (BIOXON) for aerobic mesophiles, and PDA (MCDLAB) for fungi and yeasts. Samples were collected at both 15 and 30 days of the growth cycle. The sampling process involved randomly selecting leaves from four different lettuce plants, which were then combined to form a final sample of one gram. This sample was crushed and mixed with 10 mL of sterile water. From this solution, decimal dilutions were prepared in triplicate (1:10, 1:100, and 1:1000). Of each dilution, 1 mL was added to separate Petri dishes. The respective medium was then poured into each Petri dish, allowed to solidify, and incubated. Total coliforms

Biochemical test	Medium	Incubation	Interpretation
Methyl red	EMB	48 h a 37 °C	(+) *E. coli*; (−) *Salmonella* spp.
	SS		
Indolee	EMB	2 4 h a 37 °C	(+) *E.coli*; (−) *Salmonella* spp.
	SS		
Citrate	EMB	2 4 h a 37 °C	(Blue) *Salmonella* spp.; (Green) *E.coli*
	SS		
Voges proskauer	EMB	2 4 h a 37 °C	(−) *E. coli*; (+) *Klebsiella*
	SS		
Hydrogen sulfide	EMB	2 4 h a 37 °C	(+) *Salmonella* spp.; (−) *E. coli*
	SS		
Kligler	EMB	2 4 h a 37 °C	(Ácid/Ácid) *E.coli*; (Alkaline/Ácid) *Salmonella* spp.
	SS		
Motility	EMB	2 4 h a 37 °C	(+) *E. coli* and *Salmonella* spp.
	SS		
Coagulase	MSA	2 4 h a 37 °C	(+) *S. aureus*
Catalase	MSA	N/A	(+) *S. aureus*

Table 3. Biochemical tests are conducted to confirm the identification of microorganisms and the conditions applied.

and aerobic mesophiles were incubated for 48 h at 37 °C, while fungi and yeasts were incubated for five days at the same temperature. The methodology was established according to that reported by Fernandes et al.[36] and Terrones-Fernandez et al.[37], certifying the accuracy and reliability of our results.

The disinfection percentage was calculated using Eq. (1) to determine the difference between the initial concentration (N_0) and the concentration of the irradiated sample (N_t).

$$\% \text{ disinfection} = \left(\frac{N_0 - N_t}{N_0}\right) \times 100 \tag{1}$$

where N_0 represents the CFU mL^{-1} content of the sample prior to irradiation, and N_t denotes the CFU mL^{-1} after exposure to irradiation.

Equation (2) states that bacterial inactivation occurs by dividing the concentration of bacteria in the irradiated (N_t) by the initial concentration of the sample (N_0) before UVA-LED irradiation[38].

$$\text{Inactivation Log} = Log\left(\frac{N_t}{N_0}\right) \tag{2}$$

Bacterial growth can be represented as Log N_0, where the number of colonies per mL (CFU mL^{-1}) is calculated from reproductive activity for each treatment using Eq. (3)[39].

$$Log\ N_0 = Log(N_0) \tag{3}$$

Morphological analysis

Several parameters were quantified during the experiment to assess the plant's response to UVA-LED irradiation. These variables relate to biomass determination, including leaf area (LA), dry weight (DW), and fresh weight (FW).

Leaf area (LA) was measured using a digital image analysis method with IMAGE J software[40], which evaluates leaf size by analyzing color. High-quality images were obtained using a flatbed scanner against a white background, ensuring the camera was leveled. A 20 cm transparent ruler was placed on the image to establish a reference scale. The digital images were processed in JPG format. The analysis was conducted on day 30 of the experiment, with four plants randomly selected from each treatment group.

The fresh weight (FW) was determined by randomly selecting four plants from each treatment and removing the bud from each one. The fresh leaves were then weighed using a gram balance (RHINO 215 Commercial Digital Scale, accuracy: 10 kg g^{-1}). To measure the dry weight (DW), the leaves were placed in a convection-forced heating and drying chamber (Blinder, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 70 °C for 72 h. The dry weight was recorded at the end of the experiment on day 30.

Chlorophyll was measured using the SPAD 502 PLUS SPECTRUM to quantify the absorbance of leaves in the red and near-infrared regions. The SPAD units provide a numerical value proportional to the chlorophyll content in the leaves. Measurements were taken in triplicate to ensure accuracy.

Energy consumption

Energy consumption was measured for each treatment using a STEREN wattmeter (Tlalnepantla, State of Mexico, Mexico) and was expressed in kWh.

Statistical evaluations

We conducted a one-way analysis of variance (ANOVA) alongside the least significant difference, Tukey, and Fisher tests using Minitab 17 Statistical Software (OMNITAB, Pennsylvania State University, USA). The data are presented as means with standard deviations (SD) calculated from triplicate measurements.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available because they are currently part of another ongoing research. However, the data will be made available through the corresponding author on reasonable request in appropriate time.

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Author contributions

R. E. M. E. conceived the study and developed the research design. Led the initial manuscript draft and played a significant role in the data analysis and interpretation of results. E. O. G. conducted key experiments and managed data collection. Contributed to the statistical analysis and supported the writing and revision process, focusing on the accuracy of results and interpretation. E. Z. M. provided technical expertise, overseeing the calibration and setup of LED light sources and measurement equipment. Assisted in data interpretation and reviewed the manuscript drafts to ensure clarity and accuracy. J. E. C. C. conducted the literature review and assisted in the data collection process. Provided critical feedback on data analysis and manuscript structure, contributing to the final revisions for submission. A. B. S. contributed to data interpretation and manuscript preparation. Reviewed all manuscript sections for scientific accuracy and played a central role in coordinating revisions across all authors. N. E. G. reviewed the entire manuscript for quality control and scientific rigor. Ensured adherence to journal guidelines, coordinated the final revisions, and led the submission process.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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