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UV-A Radiation Stimulates Tolerance against *Fusarium oxysporum* f. sp. *lycopersici* in Tomato Plants

Yolanda González-García [1], Diego Iván Escobar-Hernández [2], Adalberto Benavides-Mendoza [3], América Berenice Morales-Díaz [4], Emilio Olivares-Sáenz [1] and Antonio Juárez-Maldonado [5,*]

[1] Centro de Agricultura Protegida, Facultad de Agronomía, Universidad Autónoma de Nuevo León, General Escobedo 66050, Mexico; yolanda_glezg@hotmail.com (Y.G.-G.); emolivares@gmail.com (E.O.-S.)
[2] Maestría en Ciencias en Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Mexico; dieivaeh@gmail.com
[3] Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Mexico; abenmen@gmail.com
[4] Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Saltillo, Ramos Arizpe 25900, Mexico; abmoralesd@gmail.com
[5] Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Mexico
* Correspondence: antonio.juarez@uaaan.edu.mx

Abstract: UV-A radiation is a tool that has a stimulant action in plants and can be used to induce benefits through the production of bioactive compounds and the synthesis of secondary metabolites, among others. Particularly, its application could stimulate tolerance to the biotic stress caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in tomato plants; for this reason, the aim of the present study was to evaluate the ability of UV-A radiation to stimulate tolerance against Fol. For this, a tomato crop was established in which two conditions of supplementation with UV-A radiation were tested on plants inoculated with the fungus Fol. The pathogen's impact on the agronomic parameters, its incidence and severity, and the contents of stress biomarkers, secondary metabolites and photosynthetic pigments were determined. The results show that the impact of the pathogen on severity was about 60%; therefore, the impact on fruit yield was also negative (−31%). Moreover, the pathogen significantly impacted the content of stress biomarkers, and the positive control increased H_2O_2 (+23.9%), malondialdehyde (+41.7%) and proline (+54.8%). In contrast, UV-A radiation significantly decreased Fol severity (−35.5%), and prevented its negative effect on the tomato plant/fruit yield. In addition, the application of UV-A radiation decreased the contents of stress biomarkers (−10.4% $O_2^{\bullet -}$, −22% H_2O_2, and −16% MDA), and increased the contents of secondary metabolites (+13.2% flavonoids, +35% anthocyanins) and photosynthetic pigments (+17% β-carotene, +12% yellow pigments, and +19.8% total chlorophyll) in the plants inoculated with the pathogen. From the results obtained, it can be concluded that the application of UV-A radiation is a good alternative means to control the attack of pathogens such as Fol on tomato plants, without adverse consequences for the environment or the crop.

Keywords: plant pathogens; photosynthetic pigments; secondary metabolism; stress biomarkers; stress tolerance; ultraviolet radiation

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1. Introduction

Nowadays, growing food demand related to world population growth has raised enormous challenges in maintaining the productivity of crops, as well as the quality of the products. Within the range of problems that exist in agricultural production systems, one of the most important is the attack of pathogenic microorganisms such as fungi [1]. Climate change causes the negative effects of pathogens to increase, mainly due to the increase in temperatures [2]. *Fusarium oxysporum* is considered one of the best known soil-borne plant fungal pathogens, comprising more than 100 host-specific strains (special

forms), many of which have global distributions and have caused outbreaks of vascular wilt in economically important crops such as cotton (*F. oxysporum* f. sp. *vasinfectum*), banana (*F. oxysporum* f. sp. *cubense*), palm (*F. oxysporum* f. sp. *albedinis*) and tomato (*F. oxysporum* f. sp. *lycopersici*) [3]. In addition, *Fusarium* species produce three classes of mycotoxins (trichothecenes, zearalenone, and fumonisins) that can cause health risks to humans and animals [4]. *F. oxysporum* f. sp. *lycopersici* (Fol) is one of the most common diseases infecting tomato crops in both greenhouse and open field conditions, and being a soil-borne pathogen, it can survive as chlamydospores in the soil and on plant debris for long periods of time and be easily propagated via seeds and wind, causing yield losses from the seedling stage to postharvest, which range between 45 and 55% and can extend up to 70% in certain environmental conditions [5]. Therefore, it is necessary to develop new mechanisms and strategies to control the negative impacts caused by plant pathogens.

The most commonly used strategy to control pathogens is the application of chemical pesticides, mainly nitrogen-containing heterocyclic compounds, such as azoles, benzimidazoles, dithiocarbamates, dicarboximides, carbendazim, carboxin, propiconazole, benomyl and difenoconazole, which have shown effectiveness in vitro; however, only a few research articles have reported the significant control of the disease in plants through the use of these fungicides in open field or greenhouse conditions, and in addition, this practice is usually expensive [6] and can contribute to water and soil pollution [7]. These pesticides show toxicity and bio-accumulative properties, which generate adverse ecological effects, causing biological damage both in the short term (acute) and in the long term (chronic). In addition, chemical pesticides alter the food chain by modifying the habitats of non-target organisms, reducing the population of natural insect predators, altering the ecosystem's biodiversity, and promoting the dominance of invasive and unwanted species [7,8]. Moreover, their intensive usage promotes the appearance of resistant microorganisms, which increases the difficulty of controlling pathogenic microorganisms [9].

Some alternative means of control that avoid disturbances to environmental dynamics, as well as to the health of humans and animals, include the use of biopesticides derived from natural plant products, such as plant extracts, volatile organic compounds, essential oils and resins [10–12]. These contain different types of secondary metabolites of an antimicrobial nature, such as terpenoids, alkaloids and phenols; however, these metabolites are selective and/or play specific roles in different biological events, so they may not always be effective, depending on the plant species and the microorganism [13]. Biological control is another approach to the safe management of agricultural crops; for example, some strains of *Trichoderma* and *Bacillus subtilis* can significantly inhibit the spread of Fusarium wilt disease in vitro [14]. However, under field conditions, these beneficial microorganisms show high host sensitivity and specificity, as well as low profitability [15]. Breeding to induce disease resistance in tomatoes is usually highly efficient; however, the selection of specific resistance traits and the breeding process can be laborious and time-consuming, hindering progress in the commercial breeding of tomato [15]. Other strategies that have been used with some success to control bacterial and fungal crop diseases include the application of nanomaterials [16], but this has the limitation that there is little knowledge about the risks that they pose to the environment.

A little-explored alternative strategy is the use of UV radiation, which is classified into three subcategories: UV-C (100–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm) [17]. This tool shows great potential for disease control because it is a non-toxic physical method, is free of pollution, and is safe and effective for the inactivation and/or eradication of pathogenic microorganisms and toxins, without compromising the retention of nutrients, or the physical, chemical and organoleptic quality of the fruits [18]. It has been reported that UV-A and UV-B radiation can improve the quality of tomato fruits, with increases in the antioxidant capacity and the contents of bioactive compounds such as phenols and flavonoids [19]. UV radiation has been documented to positively influence several plant processes such as flowering, the accumulation of secondary metabolites, and resistance to pathogens and herbivory [20]. Specifically, UV-A radiation (315–400 nm) can have

both positive and negative effects on plant biomass and morphology, but it can also induce the production of secondary metabolites and stimulate photosynthesis [21]. It has been shown that UV-A radiation can modify plants' secondary metabolism, and therefore increase the synthesis of secondary metabolites such as flavonoids [22]. UV-A radiation has been applied postharvest and has been shown to increase the content of flavonoids such as baicalin, baicalein, wogonoside, and wogonin in *Scutellaria baicalensis* roots [23]. Moreover, secondary metabolites such as flavonoids are linked to resistance against plant pathogens [20].

Tomatoes are one of the most important vegetable crops worldwide due to their variety of uses when both fresh and processed, and are not exempt from attacks by pathogens such as *Alternaria*, *Fusarium*, *Penicillium* and *Aspergillus* [1]. *F. oxysporum* f. sp. *lycopersici* is one of the most common diseases infecting tomato crops and can cause yield losses of up to 70% [5]. For these reasons, the aim of this study was to evaluate the ability of UV-A radiation to stimulate tolerance against *Fol* in tomato plants.

2. Materials and Methods

2.1. Tomato Crop Development

A saladette-type fruiting tomato crop of indeterminate growth called "El Cid F1" (Harris Moran, Davis, CA, USA) was established in a soilless crop system with a planting density of three plants per square meter. For this, one plant was placed in a pot with a volume of 10 L of a mixture of perlite and peat moss substrates in a 1:1 ratio. The plants were set up in a tunnel-type greenhouse, with a polyethylene cover, and developed under natural lighting conditions (Figure 1A). The environmental conditions inside the greenhouse were 1100 µmol m^{-2} s^{-1} maximum radiation per day, at 15–35 °C, and a relative humidity of 30–75%. Crop nutrition was provided through a fertigation system using Steiner solution as a base [24]. The pH was adjusted to 6.5 with sulfuric acid each time the nutritive solution was prepared. The electrical conductivity (EC) of the nutritive solution was 1.9–2.5 mS cm^{-1} throughout crop development. The crop was managed to maintain a single stem and developed for 22 weeks.

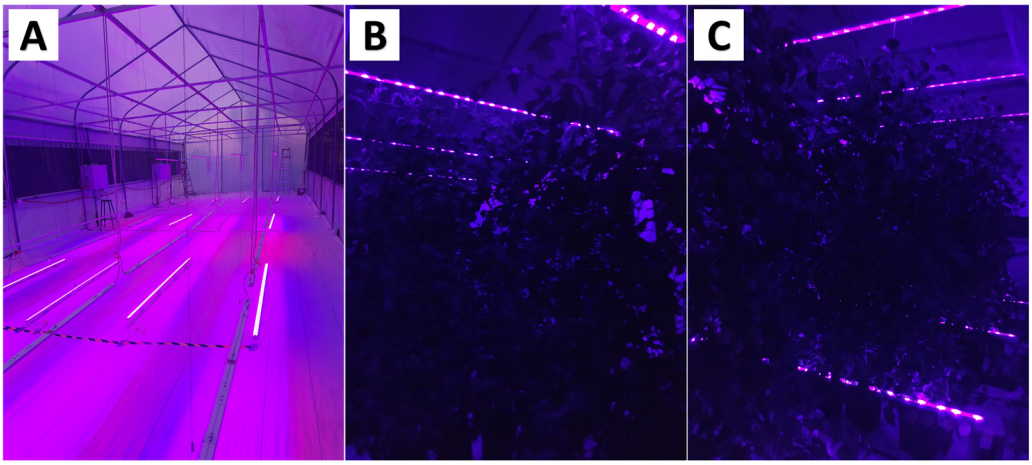

Figure 1. Greenhouse used for the experiment (**A**), and the application of treatments with supplementation of UV-A radiation via one lamp (**B**) and two lamps (**C**).

2.2. UV-A and F. oxysporum f. sp. lycopersici Treatments of Tomato Plants

The experiment was designed to evaluate the impact of supplementation with UV-A radiation on tomato plants, and its ability to stimulate tolerance against the phytopathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* race 3 (the strain was obtained from the Autonomous University of Aguascalientes, Center of Agricultural Sciences). The treatments applied were the following: (1) T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); (2) FOL, positive control (tomato plants only inoculated with Fol); (3) UV, tomato plants supplemented with UV-A radiation via one LED lamp above

of the plant canopy (Figure 1B); (4) UV + FOL, tomato plants supplemented with UV-A radiation via one LED lamp above of the plant canopy (Figure 1B) and inoculated with Fol; (5) UV2, tomato plants supplemented with UV-A radiation via one LED lamp above of the plant canopy plus one fixed lamp at the plants' bottom (Figure 1C); and (6) UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation via one LED lamp above the plant canopy plus one fixed lamp at the plants' bottom (Figure 1C).

2.3. Supplementation with UV-A Radiation on Tomato Plants

The supplementation with UV-A radiation (385 nm) was carried out with LED lamps (LILZBAL-S100WFRBPBC model, Sola Basic, Ciudad de México, Mexico) that were 2.2 m long and a had power of 100 W. The lamps were set up in two ways: (1) one lamp whose position was adjusted with the plant's growth to maintain a constant distance of 30 cm above the tomato plant canopy (Figure 1B), and (2) one lamp above the tomato plant canopy plus one fixed lamp at the bottom of the plants (Figure 1C). The greenhouse was divided into different sections to apply the different treatments. To avoid contamination with UV-A radiation, divisions were placed between each section made of white, light-impermeable polypropylene. UV-A radiation supplementation was carried out for 30 min during the night (2 h after sunset), starting 10 weeks after transplanting, and was applied every day during the rest of the tomato crop's development. The irradiation capacity of the LED lamps was 50 W m^{-2}; that is, each plant received an irradiation of approximately 16.7 W for 30 min each day when a single lamp was used, and 33.4 W when two lamps were used (Figure 1).

2.4. Inoculation of F. oxysporum f. sp. lycopersici on Tomato Plants

The tomato plants under the FOL, UV + FOL and UV2 + FOL treatments were inoculated twice with the pathogen, the first time at 6 weeks after transplanting (WAT) (at the fruit set stage). However, since the plants did not present symptoms of the disease, they were inoculated a second time at 8 WAT (at the fruit development stage).

Fol spores were cultivated at 29 °C for 15 days in petri dishes with a potato dextrose agar (PDA) medium supplied with ampicillin (100 mg mL^{-1}). Prior to inoculation, the spores were resuspended in sterile distilled water to create the working conidial suspension. The plants under treatments with Fol were inoculated with a conidial suspension of 1×10^7 spores mL^{-1}, with 200 µL of conidial suspension per plant, injected into the stem using an insulin syringe. In the first inoculation (at 6 WAT) the axil of the stem of the fifth true leaf (fifth proximal internode above ground) was penetrated with the needle to a depth of 5 mm, while in the second inoculation (at 8 WAT) the axil of the stem of the seventh true leaf (seventh proximal internode above ground) was penetrated with the needle to a depth of 5 mm.

To confirm the presence of Fol in the plants, stem isolations from the diseased plants were performed. Tissue portions (approximately 5 mm × 5 mm × 5 mm) were surface sterilized in 2% sodium hypochlorite for 1 min before being washed twice for 1 min in sterile deionized water. The portions were dried on sterile blotting paper, aseptically cut into smaller sections (approximately 2 mm × 2 mm × 2 mm) and plated into water agar. Any fungi which grew out from the isolations were sub-cultured onto potato dextrose agar (PDA), and a section of agar mounted on a slide was examined using the optical microscope to identify Fol based on its morphology.

2.5. F. oxysporum f. sp. lycopersici Incidence and Severity in Tomato Plants

Disease incidence was determined visually when the pathogen was present on the tomato plants. When the plant did not present symptoms, a value of zero was assigned, and when the plant did show disease symptoms, a value of 1 was assigned. The results are expressed as percentages. Disease severity was determined using a visual scale in each of the 12 plants per treatment, and the average of these was reported. The visual scale was according to the following scale: 0: plants indistinguishable from mock; 1 = wilting of

basal leaves (from the fifth true leaf); 2 chlorosis of basal leaves (from the fifth true leaf) and wilting of young leaves; 3 = wilting of most of the leaves, diffuse desiccation and yellowing; 4 = dead plant. Disease severity is calculated as a percentage using the following equation:

$$Disease\ severity\ \% = \frac{100 \times VS}{4} \quad (1)$$

where *VS* is the value obtained from the visual scale. The percentage severity of the disease is the mean of the 12 plants in each treatment.

2.6. Tomato Plants Agronomic Parameters

At the end of crop growth (22 WAT), the total number of harvested fruits and the fruit yield per plant, as well as the shoot and root biomass, were quantified. The shoot biomass was collected independently for each plant and placed in a drying oven to obtain the dry biomass. To obtain the root biomass, the roots of each plant were collected and washed to remove the substrate and then placed in a drying oven to obtain the dry biomass. The plant's dry weight was obtained by drying in an oven for 72 h at 70 °C.

2.7. Biochemical Parameters Measurement

The leaf samples used to carry out the different biochemical analyses were collected 17 weeks after transplanting. For sampling, the fully expanded young leaves (third or fourth leaf) were collected and placed on ice for later storage at −20 °C. The samples were lyophilized and macerated until a fine powder was obtained; using this sample, the stress biomarkers, secondary metabolites and photosynthetic pigments were determined.

2.8. Stress Biomarkers Test

Hydrogen peroxide (H_2O_2) was assessed according to the methodology described by Velikova et al. [25], and expressed as μmol g^{-1} of DW. In total, 10 mg of lyophilized sample was homogenized with 1000 μL of cold trichloroacetic acid (0.1%). The homogenate was centrifuged at 12,000× *g* for 15 min and 250 μL of the supernatant was added to 750 μL of potassium phosphate buffer 10 mM (pH 7.0) and 1000 μL of potassium iodide (1 M). The absorbance of the supernatant was read at 390 nm. The content of H_2O_2 was given on a standard.

The malondialdehyde (MDA) content was determined according to the methodology described by Velikova et al. [25] and expressed as nmol g^{-1} of DW. In total, 50 mg of lyophilized sample was homogenized in 1000 μL of thiobarbituric acid (TBA) (0.1%). The homogenate was centrifuged at 10,000× *g* for 20 min and 500 μL of the supernatant was added to 1000 μL of TBA (0.5%) in trichloroacetic acid (20%). The mixture was incubated in boiling water for 30 min, and the reaction was stopped by placing the reaction tube in an ice bath. Then, the sample was centrifuged at 10,000× *g* for 5 min, and the absorbance of supernatant was read at 532 nm. The amount of MDA–TBA complex (red pigment) was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1}.

The superoxide radical ($O_2^{\bullet -}$) content was determined according to the methodology described by Yang et al. [26] and expressed as μmol g^{-1} of DW. In total, 20 mg of lyophilized sample was added with 5 mg of PVP and homogenized with 1000 μL of cold 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 5000× *g* for 15 min at 4 °C and 600 μL of the supernatant was added to 550 μL of potassium phosphate buffer (50 mM) (pH 7.8) and 60 μL of hydroxylamine hydrochloride (10 mM). The mixture was incubated at 25 °C for 30 min. In total, 650 μL of the incubated solution was mixed with 650 μL of 3-Aminobenzenesulphonic acid (17 mM) and 650 μL of 1-Naphtylamine (7 mM). The absorbance was read at 530 nm. The content of $O_2^{\bullet -}$ was given on a standard of sodium nitrite.

Proline was determined according to the methodology described by Bates et al. [27] and expressed as μg g^{-1} of DW. In total, 50 mg of lyophilized tissue was homogenized with 1000 μL of sulfosalicylic acid (3%). The sample was then centrifuged at 15,000× *g* at

room temperature for 5 min and filtered on Whatman No. 2 paper. Subsequently, 200 µL of the filtrate was mixed with 200 µL of acid ninhydrin and 200 µL of glacial acetic acid in a test tube for 1 h at 100 °C, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 1000 µL of toluene and mixed vigorously with a test tube stirrer for 20 s. The chromophore containing toluene was aspirated from the aqueous phase and warmed to room temperature, and the absorbance was read at 520 nm using toluene as a blank. To quantify the amount of proline in the samples, an L-proline standard was used.

2.9. Secondary Metabolites Measurement

The content of total phenols was obtained according to Yu and Dahlgren [28]. In total, 100 mg of lyophilized tissue was extracted with 1 mL of a water:acetone solution (1:1) and the mixture was homogenized for 30 s. The sample tubes were centrifuged at 17,500× *g* for 10 min at 4 °C. Then, 18 µL of the supernatant, 70 µL of the Folin–Ciocalteu reagent, and 175 µL of 20% sodium carbonate (Na_2CO_3) were placed in a test tube, and 1750 µL of distilled water was added. The samples were placed in a water bath at 45 °C for 30 min. Finally, the reading was taken at a wavelength of 750 nm on the UV-Vis spectrophotometer (UNICO Spectrophotometer, Model UV2150, Dayton, NJ, USA). Total phenols were expressed in mg EQ of gallic acid per gram of DW.

The flavonoid content was determined according to Arvouet-Grand et al. [29]. For the extraction, 20 mg of lyophilized tissue was placed in a test tube to which 2 mL of reactive grade methanol was added, and this was homogenized for 30 s. The mixture was filtered using Whatman No. 1 paper. For the quantification, 1 mL of the extract and 1 mL of 2% methanolic aluminum trichloride ($AlCl_3$) solution were added to a test tube and allowed to stand for 20 min in darkness. The reading was taken at a wavelength of 415 nm on the UV-Vis spectrophotometer (UNICO Spectrophotometer, Model UV2150, Dayton, NJ, USA). The results are expressed in mg EQ of quercetin per gram of DW.

The anthocyanin content was determined according to the methodology of Lee et al. [30], and the results are expressed as mg cyanidin-3-glucoside equivalents per gram of DW. In total, 50 mg of lyophilized sample was homogenized with 2000 µL of methanol containing 1% HCl. The homogenate was centrifuged at 8000× *g* for 10 min at 4 °C. The reaction mixture consisted of 2 phases: in phase 1, 400 µL of extract was mixed with 1600 µL of 0.025 M potassium chloride (pH 1.0); and in phase 2, 400 µL of extract was mixed with 1600 µL of 0.4 M sodium acetate chloride (pH 4.5). The absorbance of both samples was read at 520 and 700 nm using methanol as the blank. The anthocyanin content was determined using the following equation:

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (2)$$

where $A = (A_{520nm} - A_{700nm})\text{pH } 1.0 - (A_{520nm} - A_{700nm})\text{pH4.5}$; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor established in D; 1 = path length in cm; ε = 26,900 molar extinction coefficient, in $L \times mol^{-1} \times cm^{-1}$, for cyanidin-3-glucoside; and 10^3 = factor for conversion from g to mg.

2.10. Photosynthetic Pigments Measurement

The contents of chlorophylls A and B (mg g^{-1} DW) and β-carotene (mg 100 g^{-1} DW) were determined according to the method of Nagata and Yamashita [31]. The chlorophyll A/B ratio was also calculated. The lyophilized sample (10 mg) was mixed with 2 mL of hexane:acetone (3:2). Subsequently, the samples were subjected to an ultrasonic bath for 5 min. They were then centrifuged at 15,000× *g* for 10 min at 4 °C. The supernatant was removed and the absorbance was read at 645 and 663 nm using a spectrophotometer. The obtained values were used in Equations (3) and (4) to calculate the chlorophyll content.

For β-carotene, absorbances at 453, 505, 645 and 663 nm were measured and the obtained values were used in Equation (5).

$$Chlorophyll\ A = 0.999 * A_{663} - 0.0989 * A_{645} \quad (3)$$

$$Chlorophyll\ B = 0.328 * A_{663} + 1.77 * A_{645} \quad (4)$$

$$\beta - carotene = 0.216 * A_{663} - 1.22 * A_{645} - 0.304 * A_{505} + 0.452 * A_{453} \quad (5)$$

Yellow carotenoids (β-carotene, β-cryptoxanthin, and zeaxanthin) and red carotenoids (capsanthin and capsorubin) were evaluated according to the method reported by Hornero-Méndez and Minguez-Mosquera [32]. The lyophilized sample (10 mg) was mixed with 2 mL of hexane:acetone (3:2). Subsequently, the samples were subjected to an ultrasonic bath for 5 min. They were then centrifuged at 15,000× *g* for 10 min at 4 °C. The supernatant was removed and the absorbance was read at 472 (yellow) and 508 (red) nm using a spectrophotometer. The obtained values were used in Equations (6) and (7) to calculate the chlorophyll content. The results are expressed as milligrams per 100 g of dry weight (mg 100 g^{-1} DW).

$$Red\ carotenoids = \frac{2144 * A_{508} - 403.3 * A_{472}}{270.9} \quad (6)$$

$$\text{Yellow carotenoids} = \frac{1724.3 * A_{472} - 2450.1 * A_{508}}{270.9} \quad (7)$$

2.11. Statistical Analysis

The experiment was undertaken using a completely randomized design. For the determination of the agronomic parameters, 12 repetitions per treatment were used considering one plant as a repetition, while for the rest of the variables evaluated, 6 repetitions per treatment were considered. In the Fol incidence and severity test, a multivariate analysis of variance was performed, as well as a Hotelling mean test ($\alpha = 0.05$). For the rest of the variables, a one-way variance analysis and a Fisher's Least Significant Difference mean test ($\alpha = 0.05$) were performed. Additionally, principal component analysis and a biplot test were performed, as well as a Pearson correlation analysis.

3. Results

3.1. Impact of UV-A Radiation on F. oxysporum f. sp. lycopersici Incidence and Severity

Stem isolations of the diseased plants demonstrated the presence of Fol in the plants inoculated with the pathogen. In addition, the diseased plants presented the typical symptoms of the disease such as wilting, chlorosis, yellowing, and necrosis.

The incidence of Fol on tomato plants did not show differences between the positive control (FOL) and the treatments with UV-A (UV + FOL, UV2 + FOL); some differences were only observed between the treatments inoculated with Fol (FOL, UV + FOL, UV2 + FOL) and those that were not inoculated (T0, UV, UV2) (Figure 2A). In contrast, from week 18 after transplanting until the end of the crop (22 weeks after transplanting (WAT)), the severity of the positive control (FOL, 60%) was significantly higher than that observed in the treatments with UV-A radiation (UV + FOL, UV2 + FOL). At 22 WAT, the UV + FOL treatment showed 35.5% decreased severity, and the UV2 + FOL treatment showed a 32.2% decrease compared with FOL (Figure 2B). The negative control (T0) and the treatments without Fol inoculation (UV and UV2) did not show any incidence or severity (Figure 2B). Figure 2C shows the impact that the inoculation of Fol had on the tomato plants (FOL), as well as the impact that supplementation with UV-A radiation had on the tomato plants inoculated with the pathogen (UV + FOL and UV2 + FOL treatments), which showed few symptoms of disease. In addition, plants under treatments without Fol did not show symptoms of the disease (T0, UV, and UV2).

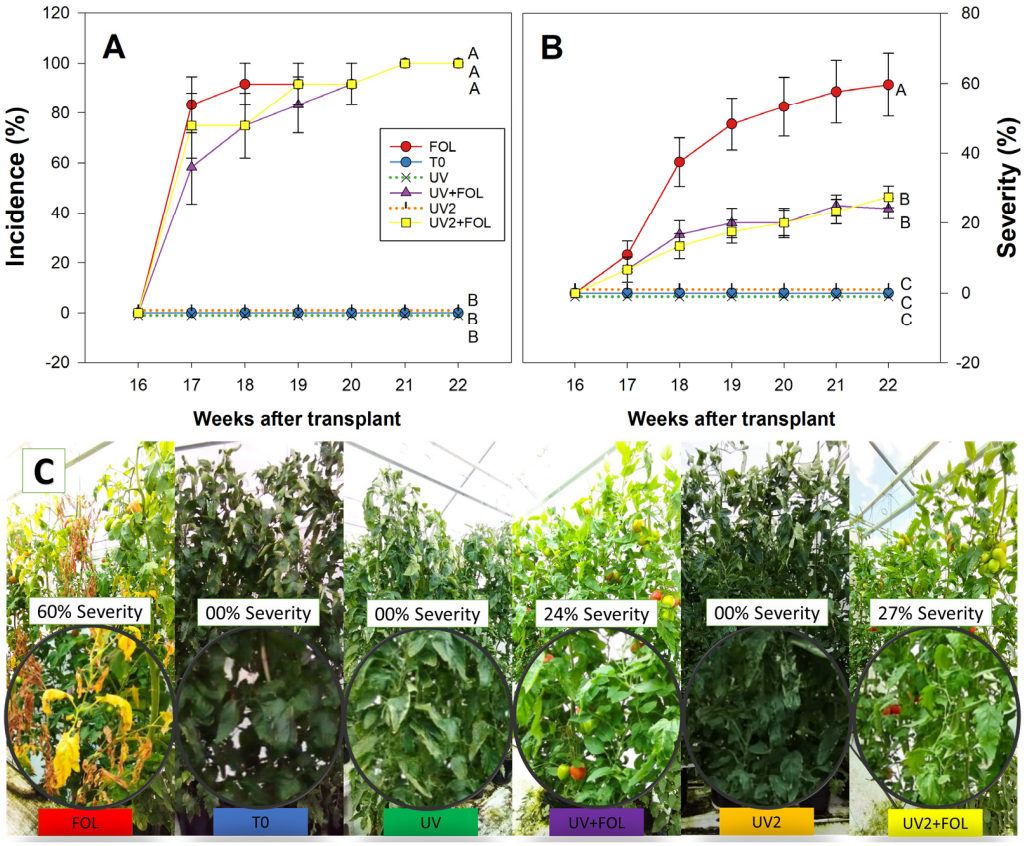

Figure 2. Impact of UV-A radiation on the (**A**) incidence and (**B**) severity of Fol in tomato plants, and (**C**) state of development of the plants at 22 weeks after transplanting. Different letters between treatments indicate significant differences according to Hotelling's test ($p < 0.05$). $n = 12 \pm$ standard error. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation via one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation via one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation via one LED lamp above the plant canopy plus one fixed lamp at the plants' bottom; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation via one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

3.2. Impacts of F. oxysporum f. sp. lycopersici and UV-A Treatments on Agronomic Parameters

The Fol treatment decreased the number of fruits per plant by 24.5% compared with the negative control (T0). Supplementation with UV-A radiation avoided this negative effect, since the results of the UV + FOL treatment were not statistically significantly different to those of the negative control (T0) (Figure 3A). This means that the growth of tomato plants was significantly affected by Fol; however, supplementation with UV-A radiation managed to reduce the negative impacts caused by the pathogen.

Fol negatively affected the fruit yield of tomato plants by −31% compared with T0. However, supplementation with UV-A radiation caused the results of UV + FOL treatment to be not statistically significantly different from those of T0, and the former also resulted in 26% higher fruit yield than FOL (Figure 3B).

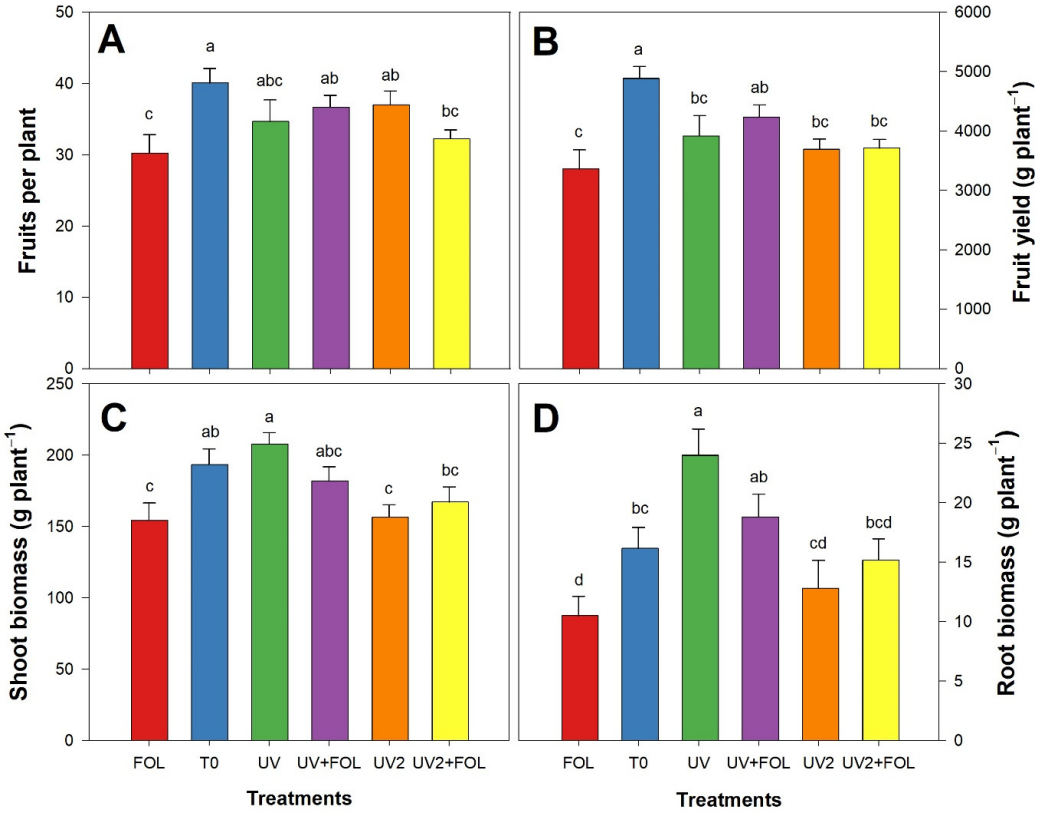

Figure 3. Impact of UV-A radiation on (**A**) Fruits per plant, (**B**) Fruit yield, (**C**) Shoot biomass, and (**D**) Root biomass of tomato plants inoculated with Fol obtained at 22 weeks after transplanting. Different letters between treatments indicate significant differences according to Fisher's Least Significant Difference test ($p < 0.05$). $n = 6 \pm$ standard error. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation through one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation through one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

The biomass of tomato plants inoculated with Fol was also negatively affected; shoot biomass decreased by 20% while root biomass decreased by 35% compared with T0. However, supplementation with UV-A radiation negated this negative effect of the pathogen on the biomass of tomato plants, since the results of the UV + FOL treatment were not statistically significantly different from those of T0 (Figure 3A,B). In addition, it was observed that UV-A radiation stimulated biomass production in healthy plants; the root biomass was 48.4% greater in the UV treatment than in T0 (Figure 3D).

3.3. Impacts of F. oxysporum f. sp. lycopersici and UV-A Treatments on Stress Biomarkers

As regards the superoxide radical, a positive effect was only observed under the UV + FOL treatment, which presented a 10.4% reduced content of this radical compared with FOL, while the results of rest of the treatments were equal (Figure 4A).

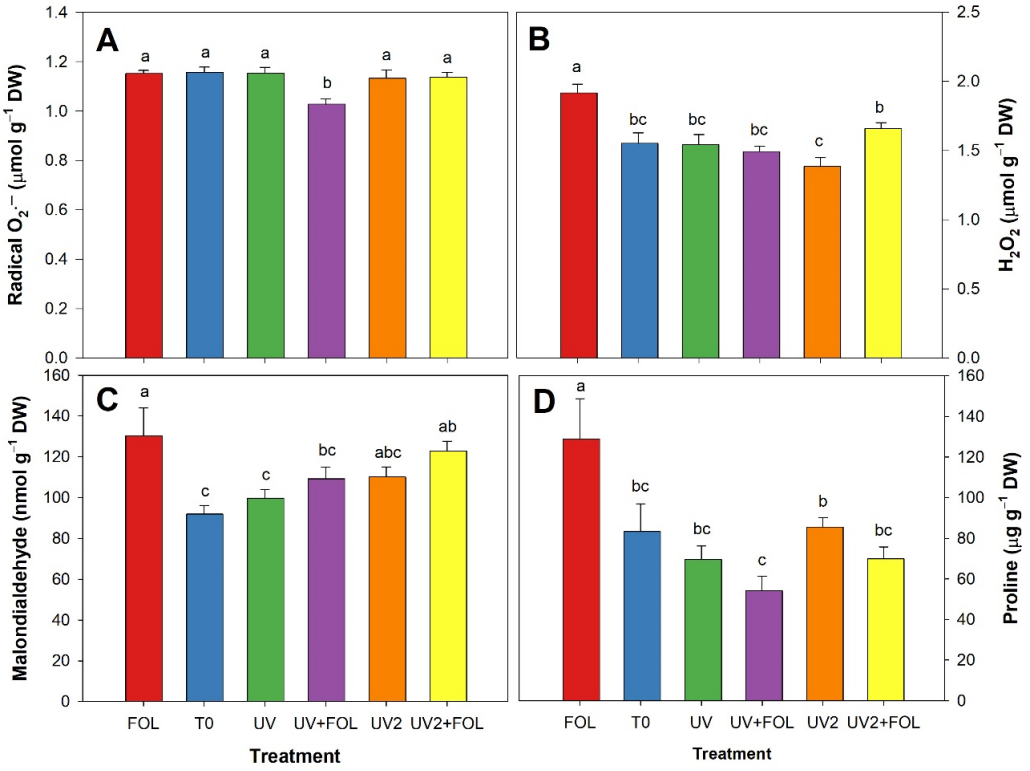

Figure 4. Stress biomarkers in leaves of tomato plants inoculated with Fol and supplemented with UV-A radiation. (**A**) Superoxide radical, (**B**) Hydrogen peroxide, (**C**) Malondialdehyde, and (**D**) Proline. The leaf samples (fully expanded young leaves) were collected 17 weeks after transplanting. Different letters between treatments indicate significant differences according to Fisher´s Least Significant Difference test ($p < 0.05$). $n = 6 \pm$ standard error. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation through one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation through one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation through one LED lamp above of the plant canopy plus one fixed lamp at the plants' bottom; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

The Fol treatment significantly increased the hydrogen peroxide content; 23.9% more H_2O_2 was observed in FOL compared with T0. However, the supplementation with UV-A radiation prevented the accumulation of H_2O_2 in the plants inoculated with the pathogen; the results of both UV + FOL and UV2 + FOL treatments were not statistically significantly different from those of T0 (Figure 4B).

The MDA content increased significantly in FOL, where 41.7% more content was observed compared with T0. However, the UV + FOL treatment prevented the production of MDA, the content of which was not statistically significantly different here from that in T0 (Figure 4C).

The proline content was higher in FOL as the inoculation with Fol induced a 54.8% greater accumulation than in T0. However, supplementation with UV-A radiation decreased proline accumulation in plants inoculated with the pathogen; UV + FOL showed a decrease of 58%, while UV2 + FOL showed a decrease of 45.8%, compared with T0 (Figure 4D).

These results show that the negative impact of Fol on tomato plants was also reflected at the biochemical level, where a significant increase was observed in most of the stress biomarkers (Figure 4).

3.4. Impacts of F. oxysporum f. sp. lycopersici and UV-A Treatments on Secondary Metabolites

As regards the content of secondary metabolites, it was observed that inoculation with Fol (FOL) induced the lowest content of flavonoids in tomato plants; however, the effect was not significant compared with T0. In contrast, it was observed that UV-A radiation induced the accumulation of these metabolites in both healthy plants and those inoculated with the pathogen; UV2 treatment led to the presence of 11.6% more flavonoids than in T0, while UV + FOL treatment led to the presence of 13.2% more than in FOL (Figure 5A).

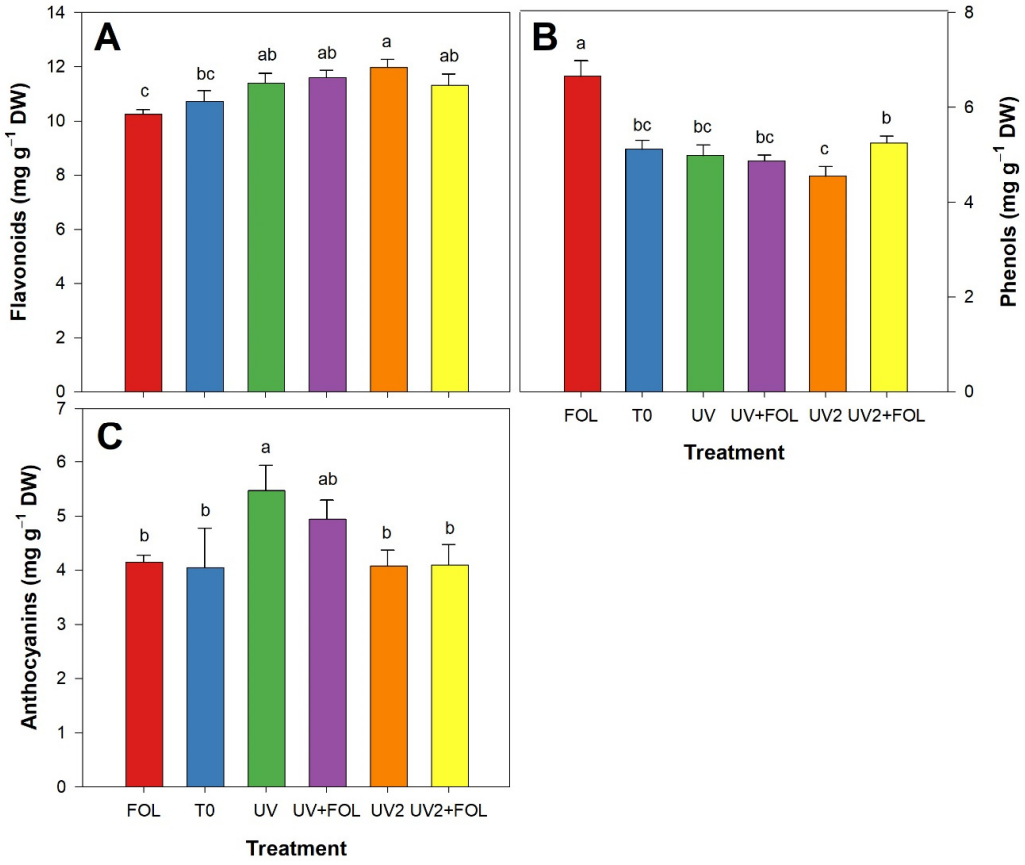

Figure 5. Contents of secondary metabolites in leaves of tomato plants inoculated with Fol and supplemented with UV-A radiation. (**A**) Flavonoids, (**B**) Phenols, and (**C**) Anthocyanins. The leaf samples (fully expanded young leaves) were collected 17 weeks after transplanting. Different letters between treatments indicate significant differences according to Fisher´s Least Significant Difference test ($p < 0.05$). $n = 6 \pm$ standard error. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation through one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation through one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

The phenols content increased significantly due to the effect of Fol; it was observed that FOL led to a 30% greater content than in T0. Supplementation with UV-A radiation negated the observed effect of inoculation with Fol, since the UV + FOL and UV2 + FOL treatments yielded equal results to T0, and led to a significantly lower phenols content than in FOL (Figure 5B).

The anthocyanin content was not modified by inoculation with Fol; the FOL content was statistically equal to that under T0. However, it was observed that UV-A radiation in

healthy tomato plants induced the accumulation of these metabolites, as UV treatment led to 35% more anthocyanin than in T0 (Figure 5C).

3.5. Impacts of F. oxysporum f. sp. lycopersici and UV-A Treatments on Photosynthetic Pigments

The β-carotene content was not modified by the presence of the pathogen. However, a significant increase in this pigment was observed due to the effect of UV-A radiation in plants inoculated with Fol; the UV2 + FOL treatment presented approximately 17% more β-carotene compared with both T0 and FOL (Figure 6A). Similarly, the contents of yellow pigments were increased by the UV2 + FOL treatment—by 11% and 12% compared with T0 and FOL, respectively. Fol by itself did not affect the content of yellow pigments (Figure 6B). In contrast, the content of red pigments did not present differences between treatments (Figure 6C).

The inoculation with Fol consistently induced a lower chlorophyll content; however, the contents of chlorophyll A presented significant differences with respect to T0 (−10%). In contrast, supplementation with UV-A radiation induced an increase in chlorophyll A—the UV2 + FOL and UV + FOL treatments led to 20.5% and 10.6% increases, respectively, compared with FOL (Figure 6D). The chlorophyll B content was not affected by FOL compared with T0; however, the UV2 + FOL treatment led to a significant increase in this pigment compared with FOL (+17.7%) (Figure 6E). The total chlorophyll content was increased under the UV2 + FOL treatment compared with FOL (+19.8%) (Figure 6F). The chlorophyll A/B ratio was affected by the treatments (Figure 6G); the UV treatment manifested the largest ratio (2.39) followed by T0 (2.25). The UV2 treatment presented the lowest chlorophyll A/B ratio (2.12).

These results show a consistently negative impact of Fol on photosynthetic pigments' contents in tomato plants (Figure 6).

3.6. Principal Component Analysis and Correlations

The interactions between the treatments and the response variables in tomato plants inoculated with Fol and supplemented with UV-A radiation are presented in a biplot in Figure 7A. This figure explains 68.97% of the total variability in the treatments according to the response variables. The variables evaluated clearly form three groups, due to the high correlations between them. In the largest group (group 1, green circle) are flavonoids, red pigments, number of fruits, fruit yield, shoot and root biomass, and anthocyanins. On the opposite side (group 2, red circle) is a group formed by the incidence and severity, MDA, H_2O_2, $O_2^{\bullet -}$, and phenols, which presents a negative correlation with the variables of group 1. In the third group (yellow circle), there are chlorophylls, yellow pigments and β-carotene.

It is interesting to note that the variables of group 1 are associated with UV, UV2, UV + FOL, and T0 treatments, these being the treatments yielding the best agronomic characteristics in tomato plants. In contrast, group 2 is associated with plants inoculated with Fol (FOL). This group had the greatest effects on the plants' agronomic characteristics and showed the highest concentration of stress biomarkers (H_2O_2 and MDA). Finally, in group 3, a clear association can be observed between the UV2 + FOL treatment and the contents of chlorophylls and yellow pigments, including β-carotene, which is confirmed by the analysis of variance presented herein (Figure 6).

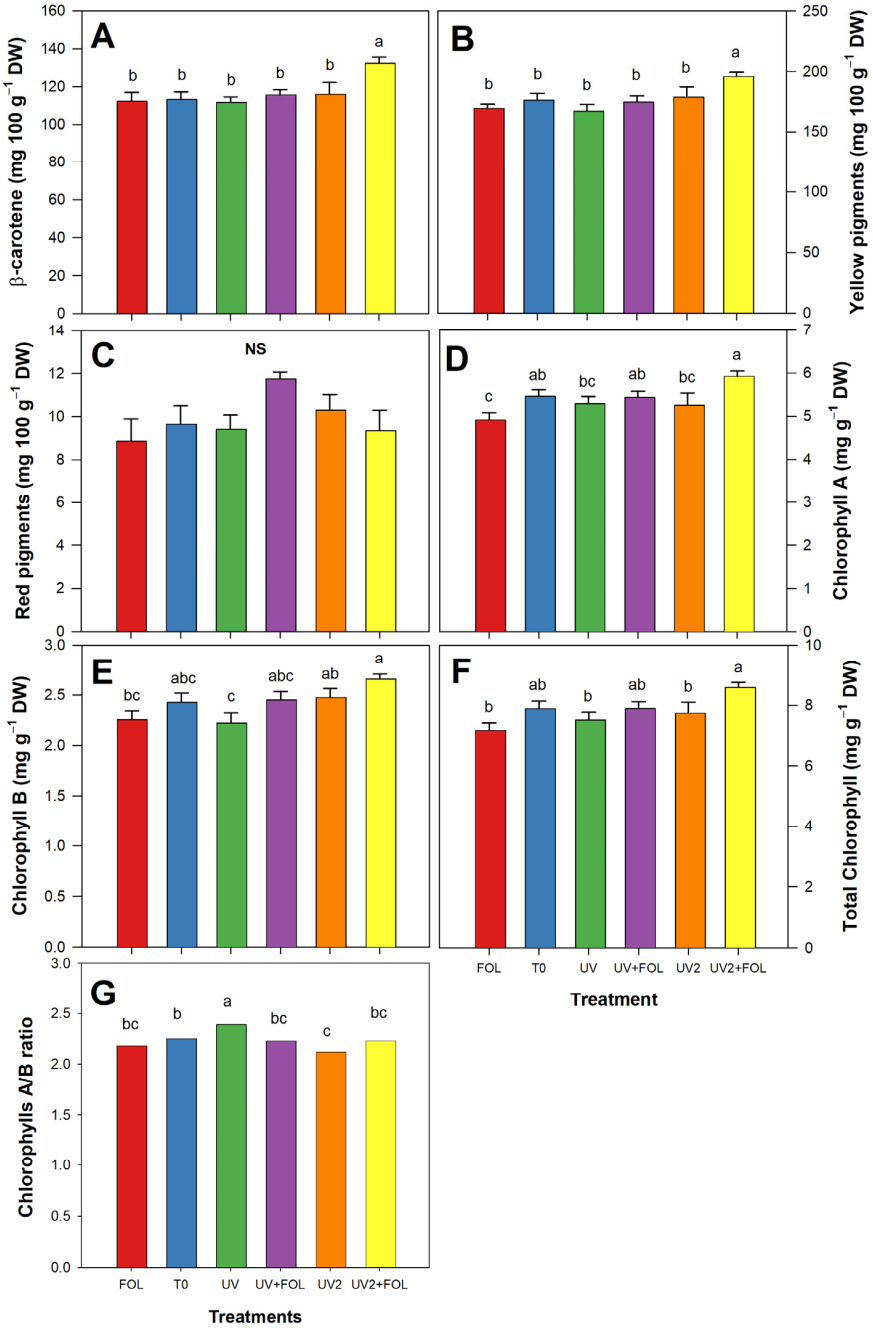

Figure 6. Photosynthetic pigment contents in leaves of tomato plants inoculated with Fol and supplemented with UV-A radiation. (**A**) β-carotene, (**B**) Yellow pigments, (**C**) Red pigments, (**D**) Chlorophyll (**A**,**E**) Chlorophyll (**B**,**F**) Total Chlorophyll, and (**G**) Chlorophylls A/B ratio. The leaf samples (fully expanded young leaves) were collected 17 weeks after transplanting. Different letters between treatments indicate significant differences according to Fisher´s Least Significant Difference test ($p < 0.05$). $n = 6 \pm$ standard error. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation through one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation through one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

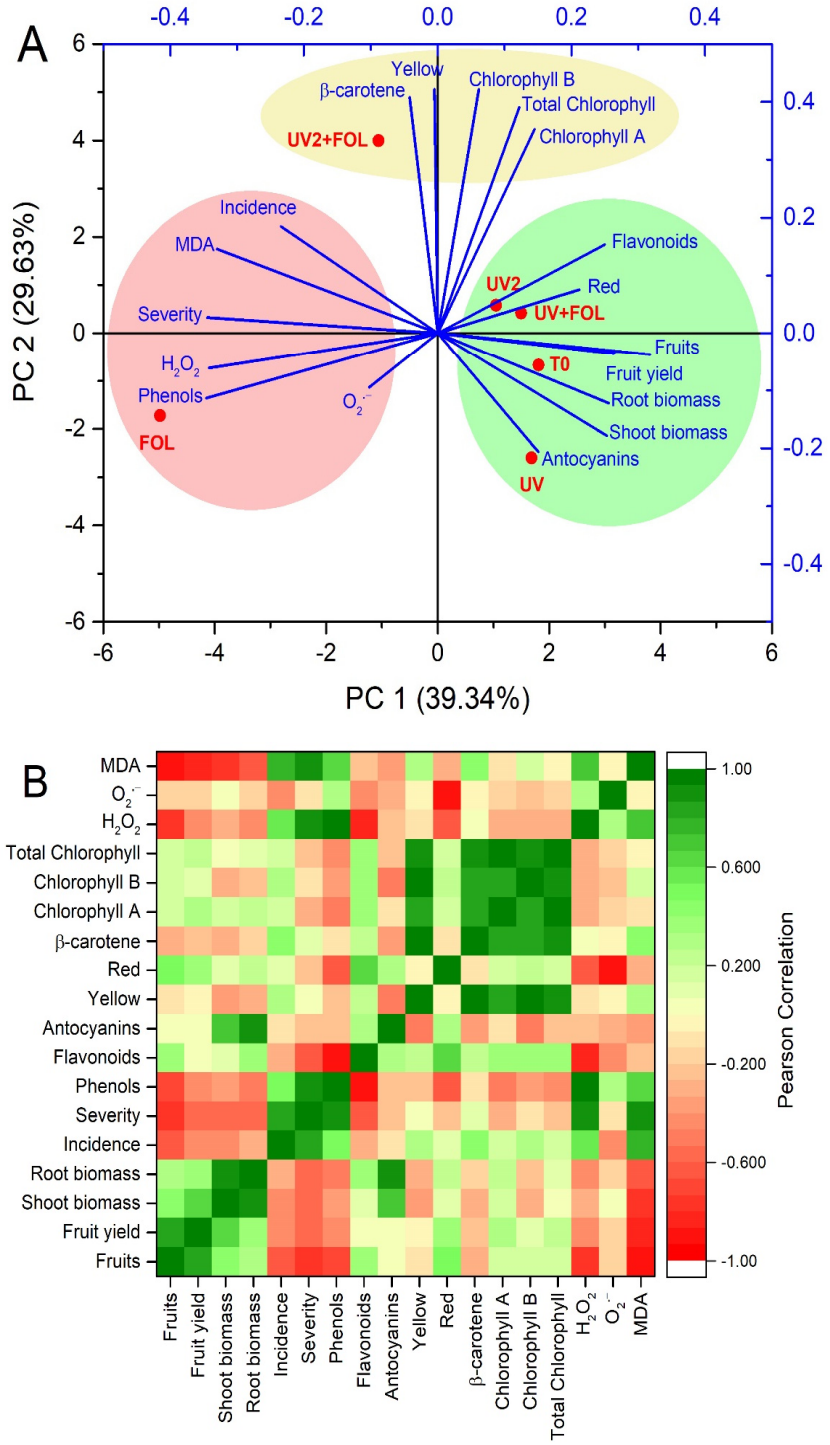

Figure 7. Biplot of the treatments and response variables (**A**), and Pearson correlations of the response variables (**B**) of tomato plants inoculated with Fol and supplemented with UV-A radiation. T0, negative control (tomato plants without UV-A radiation and without Fol inoculation); FOL, positive control (tomato plants only inoculated with Fol); UV, tomato plants supplemented with UV-A radiation through one LED lamp; UV + FOL, tomato plants supplemented with UV-A radiation through one LED lamp and inoculated with Fol; UV2, tomato plants supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants; and UV2 + FOL, tomato plants inoculated with Fol and supplemented with UV-A radiation through one LED lamp above the plant canopy plus one fixed lamp at the bottom of the plants.

Figure 7B shows the correlation between all the variables evaluated. Here, we can confirm a negative correlation between the agronomic characteristics of the tomato plants (shoot and root biomass, fruit yield, and fruits) and stress biomarkers (H_2O_2, $O_2^{\bullet -}$, and MDA). In addition, a clear negative correlation can be observed between the agronomic parameters and the Fol incidence and severity, as well as the phenol content. As expected, a positive correlation can be observed between stress biomarkers (H_2O_2 and MDA) and Fol severity and phenol content. Moreover, a positive correlation can be observed between chlorophylls, yellow pigments and β-carotene.

4. Discussion

The results of this study show that the application of UV-A radiation (through one or two lamps) was effective in reducing the severity of disease caused by Fol (Figure 2), which was beneficial in terms of fruit yield and other agronomic parameters of plants infested with the pathogen (Figure 3). However, it is also possible to increase the net photosynthesis rate (Pr) with UV-A supplementation, and thus potentially improve plant agronomic parameters. This was reported by Samuoliene et al. [33] in red leaf lettuce treated with different UV-A wavelengths (402, 387 and 367 nm). They observed an increase in Pr when they supplemented the plants with UV-A radiation; however, the authors propose that this could be due to the increase in stomatal conductance.

The impact of UV radiation on microorganisms manifests in the first instance through the direct effects of several mechanisms that have been described, and which result in the inhibition or death of microorganisms. UV radiation easily damages the DNA of eukaryotic cells, which induces the formation of cytotoxic photoproducts known as cyclobutanopyrimidine dimers (CPD) and (6-4)-pyrimidine-pyrimidone (6-4PP), which are highly damaging to cells. Specifically, UV-A radiation also causes inhibition of the Phr1, Phr2 and CryA photolyase enzymes responsible for the restoration of DNA damaged by UV's effects and the recovery of cell viability (photoreactivation) [34,35]. In addition, this hinders the regulation of the formation of reproductive structures, as well the secondary metabolism of pathogenic microorganisms [36]. Furthermore, UV-A radiation can cause death or inactivation in microorganisms by generating stress. This is because it can excite the thiouridine residues present in transfer RNA (tRNA), leading to growth arrest as a response pathway. Moreover, it generates toxic effects through the excitation of chromophores such as B2 (riboflavin) and K3 (menadione), or their byproducts; these can aggregate in cellular environments and become photosensitized to produce cytotoxicity through the production of reactive oxygen species (ROS), which result in cell lethality [37,38].

UV radiation is not selective in impacting only pathogenic microorganisms. In plants, the production of ROS due to the effects of excessive UV radiation generates damage to the DNA, proteins, lipids and cell membrane, especially in the mitochondria, nucleus, chloroplasts and apoplast [39]. Hence, in our study, higher contents of β-carotene and yellow pigments (Figure 6A,B) were observed when two lamps were used to apply UV-A radiation, since these pigments can neutralize ROS [40]. However, in moderate doses, the ROS products have a biostimulant function in plants, acting as signaling molecules, and thus instantly activate defense mechanisms to synthesize enzymatic and non-enzymatic secondary metabolites, which have a bactericidal and/or fungicidal nature, providing protection to plants against pathogenic microorganisms [39,41]. Our results were consistent when UV-A radiation was applied via one or two lamps; the flavonoids content increased regardless of whether the plants were inoculated with Fol or not (Figure 5A). In the case of anthocyanins, a positive effect was only observed when a single lamp was used to apply UV-A radiation (Figure 5C). Vodnik et al. [42] evaluated the effects of two wavelengths of UV-A (365 and 385 nm) in green leaf basil (*Ocimum basilicum* L.). The authors did not observe negative effects on plant growth or photosynthetic functions—they only observed that UV-A supplementation increased the contents of some phenolic compound derivatives of rosmarinic acid and caffeic acid (CAFA; RA-glucoside, CAFA-glucoside). Lee et al. [43] did not observe negative effects related to UV-A (peak wavelength 375 nm) supplementation in

two varieties of lettuce (*Lactuca sativa*, cv. red-leaf "New Red Fire" and green-leaf "Two Star") in terms of shoot fresh mass, leaf area, or leaf number; however, they observed a decrease in dry biomass.

UV-A radiation has been studied to a greater extent in biomedical fields because it has been observed to possess germicidal properties, which are attributed to the inhibitory effect it has on microorganisms resistant to physical sterilization methods, such as *Enterococcus faecalis*, *Bacillus atrophaeus* endospores and lentivirus [38]. However, it also has great potential for use in the control of pathogenic microorganisms of horticultural crops, since the mechanisms of action are essentially the same [44].

The results of this work are consistent with those reported by other authors. Suthaparan et al. [45] inoculated cucumber plants (*Cucumis sativus*) with the fungal pathogen *Podosphaera xanthii;* the plants were irradiated for 2 h at night with UV-A + UV-B radiation. The authors observed that 12 days after the germination and development of conidia and colonies, the severity of the fungus decreased. Cohrs and Schumacher [35] exposed *Botrytis cinerea* conidia in vitro to UV-C light for 6 and 8 min and observed a decrease in the growth of hyphae and mycelium, and a reduction in the photoreactivation capacity due to the inhibition of *BcCRY2* gene expression of the *CryA* family. Additionally, they tested the severity of the fungus on bean (*Phaseolus vulgaris*) leaves by inoculating them with mycelium discs and conidial suspension, and reported a reduced radial growth rate and a delay in the onset of conidial growth [35]. Hayes et al. [44] evaluated in vitro the effect of UV-C on *Rasoltonia solanacearumse* and observed a decrease in surviving bacterial colony-forming units (CFU) to below the detection limit after 5 min of exposure to radiation.

As well as being used in pathogen control, supplementation with UV-A radiation can be a valuable tool for promoting seedling growth and vigor since it is able to enhance physiological processes such as germination, growth, and biomass accumulation in various crops, making the photosynthesis process more efficient; therefore, some UV-A wavelengths are used in this process in the visible light regions (>400 nm) [41]. In this study, the impact of UV-A radiation on the agronomic parameters of plants inoculated with Fol was positive, mainly when a single lamp was used for application. It was observed that the number of fruits per plant, fruit yield per plant and root biomass were significantly improved here compared with control plants inoculated with Fol (Figure 3A,B,D). In contrast, the application of UV-A radiation with two lamps only had a positive effect on the number of fruits per plant in plants inoculated with Fol (Figure 3A); however, this did not modify the fruit yield per plant (Figure 3B). UV-A radiation induces the upregulation of genes involved in photosynthetic efficiency that encode the protein subunits of photosystem II (PSII), D1 (*psbA*) and CP47 (*psbB*) proteins [46]. It has also been shown that UV-A radiation can stimulate leaf growth because it promotes cell division, expansion and elongation, and increases the thickness of the palisade parenchyma and the epidermis [21]. However, it can also induce negative effects, such as reducing dry biomass, as was observed in two varieties of lettuce (*L. sativa*, cv. red-leaf "New Red Fire" and green-leaf "Two Star") [43]. The plant's response depends on the light wavelength, intensity and period of exposure; 5 min or more of exposure generates positive effects, while detrimental effects appear after 6 h, suggesting a hormesis effect [17,46].

Mariz-Ponte et al. [47] irradiated tomato seeds (*Solanum lycopersicum* L. cv. Oxheart) with UV-A radiation for 2 h and reported an increase in germination, shoot length, cotyledon area, and dry matter content. Jin-Hui et al. [41] reported an increase in the fresh and dry weights of kale (*Brassica oleracea* var. acephala) plant shoots and roots which were irradiated with two types of UV-A LEDs (370 and 385 nm) for 5 days. In the same species, Choi et al. [48] demonstrated an increase in leaf area and root biomass when irradiating plants with three types of UV-A LEDs (375, 385 and 385 nm) for 7 days. The aforementioned effects can have a positive impact on plants diseased with pathogens through a greater capacity for growth and development, as observed in the present study, thus resulting in a decrease in pathogen severity in the plants.

Filamentous fungi such as Fol include three subfamilies of NADPH oxidases, NoxA, NoxB and NoxC, which produce ROS in infested plant cells—mainly superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). These are responsible for the regulation of redox signals that control cellulose degradation, toxicity, spore formation and the proliferation of the pathogen, which cause cell death in the host [49]. Clearly, UV-A radiation did not negatively impact ROS production in tomato plants (Figure 4A,B), but it did affect the control of Fol severity (Figure 2B); it was observed that the superoxide radical decreased under the UV + FOL treatment, while the rest of the treatments showed no significant effects (Figure 4A). As for H_2O_2, an even greater tendency was observed, since supplementation with UV-A radiation prevented the accumulation of H_2O_2 in plants inoculated with the pathogen (Figure 4B). This seems to indicate that UV-A radiation could have an effect on the activity of Fol's NADPH oxidases, which would prevent the damage caused by this pathogen in tomato plants.

ROS play a dual role in organisms, since they are not only the cause of cellular lipid peroxidation, but also act as signaling molecules and activators of secondary antioxidant metabolites that inhibit their oxidative damage [50]. The production of ROS, either by the pathogen or by the UV effect, can cause an increase in the peroxidation levels of the membrane lipids, increasing the MDA content [51]. In the present study, the positive control (FOL) and the UV2 + FOL treatment led to the highest MDA content, which indicates a clear detrimental effect on the plants as exerted by the pathogen; in addition, a hormetic effect has been observed following supplementation with UV-A radiation [17]. The results concerning the proline content are also consistent; this amino acid is an important osmolyte that functions as an ROS scavenger, as well as a carbon and nitrogen reserve in stressed plants, helping to stabilize cell structures and prevent lipid peroxidation [52]. In addition, proline can protect cells against the damage induced by UV-A radiation [53]. Therefore, the results show that supplementation with UV-A radiation did not generate stress in tomato plants, unlike inoculation with Fol (Figure 4).

Ultraviolet radiation is captured in plants by photoreceptors (cryptochromes and phototropins), which can activate the biosynthesis of specific secondary metabolites such as hydroxycinnamic acids, flavonoids (diaryl-propane phenolic compounds) and anthocyanins, which serve to protect against the sun [54]. Hence, most phenolic compounds, including flavonoids and anthocyanins, can absorb UV-A in the range of 315 to 400 nm [39,55]. Our results are consistent with this; when UV-A radiation was applied, the flavonoid content increased regardless of whether the plants were inoculated with Fol or not (Figure 5A). Anthocyanins were increased only when a single lamp was used to apply UV-A radiation (Figure 5C). Chen et al. [56] demonstrated an increase in flavonoids and anthocyanins in lettuce plants (*L. sativa* L. cv. Klee) irradiated with UV-A radiation for 30 min over a period of 5 days. Miao et al. [23] applied UV-A radiation to *Scutellaria baicalensis* plants for 8 h postharvest and reported a significant increase in the contents of total flavonoids in addition to the specific flavonoids baicalin, baicalein, wogonoside and wogonin. Vodnik et al. [42] reported that UV-A (365 and 385 nm) supplementation increased the contents of some phenolic compound derivatives of rosmarinic acid and caffeic acid in green leaf basil (*O. basilicum* L.). These compounds have excellent antioxidant activities, possess the ability to promote defense against crop pests and diseases, and increase the nutritional quality and organoleptic attributes of the edible parts [17]. Hence, supplementation with UV-A radiation may indirectly stimulate tolerance against Fol (as seen here, Figure 2) via the production of these secondary metabolites. However, a decrease in phenols and flavonoids has also been reported in two lettuce varieties (*L. sativa* cv. red-leaf "New Red Fire" and green-leaf "Two Star") supplemented with UV-A radiation [43].

The levels of photosynthetic pigments (chlorophylls and carotenoids) are closely related to the photosynthetic capacity of higher plants under environmental stress. It has been shown that a high content of chlorophylls can allow the absorption of more light energy, and therefore result in a higher photosynthetic capacity [57]. In addition, the chlorophyll A/B ratio has also been used as an indicator of plant stress [58,59]. A

high chlorophyll A/B ratio is related to a greater ability to tolerate stress [59]. Our results indicate a higher A/B ratio with UV treatment (Figure 6G), indicating that the application of UV-A radiation can increase stress tolerance, as observed in this study. In contrast, it was observed that the UV-2 treatment led to the lowest A/B ratio (Figure 6G), which may indicate that the application of UV-A radiation with two LED lamps could be excessive. Accessory pigments such as β-carotene and lutein absorb specific wavelengths to make photosynthesis more efficient, and also neutralize the singlet oxygen generated in this process, while lutein can also quench chlorophyll in the triplet state, thus preventing the formation of ROS [40]. In our study, an increase in β-carotene and yellow pigments was only observed with the UV2 treatment (Figure 6A), which may indicate an adjustment of the plant's ability to tolerate the possible stress caused by an excess of UV-A radiation.

Some works have reported that UV-A supplementation increases plants' photosynthetic pigments. Biswas et al. [60] reported an increase in chlorophyll A in *Arabidopsis thaliana* plants irradiated with UV-A for 10 days under in vitro conditions. Badmus et al. [61] reported in *A. thaliana* leaves an increase in the carotenoids anteraxanthin, neoxanthin, violaxanthin and lutein when plants were exposed to UV-A radiation for a period of 3.75 h during the day, and showed that their accumulation does not depend on a particular photoreceptor (cryptochrome and phototropin). Lee et al. [43] reported an increase in the chlorophyll and carotenoid contents in the leaves of two varieties of lettuce (*L. sativa*, cv. red-leaf "New Red Fire" and green-leaf "Two Star") supplemented with UV-A radiation. These results are consistent with those obtained in the present study. Moreover, due to the potential benefits derived from the production of photosynthetic pigments, it can be concluded that UV-A radiation induces positive effects in tomato plants inoculated with Fol.

5. Conclusions

F. oxysporum f. sp. *lycopersici* is a pathogen that causes severe damage in tomato plants. In this work, the disease pressure showed about 60% severity, and therefore, the negative impacts on agronomic parameters were also significant. Moreover, the pathogen significantly increased the contents of stress biomarkers, demonstrating a negative impact at the biochemical level.

In contrast, supplementation with UV-A radiation was able to significantly reverse this negative impact. UV-A radiation decreased Fol severity and the contents of stress biomarkers, which had a positive effect on the growth and development of the tomato plants. Furthermore, UV-A radiation had a positive impact on the production of secondary metabolites—mainly flavonoids and anthocyanins. These metabolites are associated with the tolerance of plants against pathogenic microorganisms, including fungi such as Fol, indicating that UV-A radiation could induce tolerance against this pathogen indirectly through the synthesis of secondary metabolites. The contents of photosynthetic pigments also increased thanks to the UV-A radiation; both photosynthetic pigments and the secondary metabolites contribute to the neutralization of ROS and, therefore, to reducing the negative impacts caused by Fol.

According to the results, using a single UV lamp positioned above the plant canopy is the best means to induce tolerance against Fol and produce the best responses in the agronomic parameters of tomato plants.

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