



Article

Physiological Study of the Efficacy of Archer[®] Eclipse in the Protection against Sunburn in Cucumber Plants

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Abstract: Sunburn is an important issue affecting the yield of many crops, mainly in arid and semi-arid regions. Excessive solar radiation and high temperatures can reduce growth and cause leaf chlorosis, oxidative stress, and photosynthesis impairment. It is thus necessary to develop agricultural techniques to protect plants in a cost-effective and reproducible manner. A potential method is through the spray of protective compounds based on particulate films, such as those based on kaolin. The objective of this study is to evaluate the effects of spraying the protective product Archer[®] Eclipse, created by Atlántica Agrícola S.A. (Alicante, Spain), on sunburn damage in a sensitive species such as the cucumber plants (*Cucumis sativus* L.). To evaluate the effects of sunburn on the plants, parameters related to biomass, leaf temperature, photosynthesis, and oxidative stress were analysed. Plants sprayed with Archer[®] Eclipse showed fewer sunburn symptoms and obtained 43% more shoot biomass than those that were not treated. In addition, plants sprayed with Archer[®] Eclipse showed 3 °C lower leaf temperatures, higher photosynthesis performance, 88% more water use efficiency, and 21% more chlorophyll concentration. Finally, plants treated with Archer[®] Eclipse presented 6% less accumulations of carotenoids and 67% less total phenols, but lower oxidative stress indicators. In conclusion, this study confirms the efficiency of Archer[®] Eclipse in protecting a sensitive vegetable plant such as the cucumber from sunburn-inducing conditions.

Keywords: *Cucumis sativus*; kaolin; sun protector; reactive oxygen species; photo-oxidative stress



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1. Introduction

Injuries caused by what is commonly referred to as “sunburn” on many plants are major problems that affect the yield of many crops, mainly in arid and semi-arid regions [1]. These areas are characterised by high solar radiation and elevated temperatures during the crops’ growing season [2]. In addition, in the coming years, climate change will increase both temperature and drought and this, in turn, will increase the frequency of sunburn in plants, bringing about a loss in terms of production and profits in agricultural systems [2].

Solar damage in plant tissues, caused by excessively high temperatures and light irradiation, generates photo-oxidative stress due to the high accumulation of ROS (a phenomenon called photo-oxidation) and leads to the manifestation of typical sunburn symptoms [3]. These symptoms include tissue discoloration, yellowing, browning, and necrosis in cases of severe damage [4]. Chlorophyll (Chl) concentrations decrease, while other pigments, such as xanthophyll carotenoids, increase, leading to the symptoms being visualised [4]. Indeed, the Chl content of leaves was one of the most important physiological characteristics that

were altered under this type of stress, and it is considered one of the most important indicators of sunburn in plants [4]. Furthermore, under environmental conditions of sunburn, a loss of Chl causes a reduction in light absorption by the plant and, therefore, the generation of ROS and photo-oxidative stress [1]. On the other hand, some defence mechanisms against photo-oxidative stress described in plants have been: (i) a change in the redox state and (ii) the regulation and induction of antioxidant metabolites by increasing the activity of antioxidant enzymes and the accumulation of phenolic compounds that act as protectors [5].

To mitigate “sunburn” effects, it is necessary to develop and implement the use of agricultural techniques to protect plants from both extreme solar radiation and high temperatures, doing so at a low financial cost. Some solutions include the use of coloured nets to cast shade on crops and sprinkler irrigation systems that cool the crops to counteract the effects of high summer temperatures [6–8]. However, some problems with these systems are that they require a large amount of technology, the availability of good quality water, and they are expensive, as well as having a secondary effect on the spread and appearance of some diseases.

In recent years, the direct application to plants of protective compounds, mostly based on the use of particle films, has been proposed as a new tool that is useful and effective in the prevention of sunburn [9]. Thus, several studies have delved into this technique in different crops, confirming the effectiveness of these compounds in protecting plant tissues, leaves, and fruits from extreme solar radiation and high temperatures [10].

In terms of the protective compounds based on the use of particulate films, one of the most widely used is kaolin, which consists of a crumbly clay that is rich in the mineral kaolinite, whose chemical composition is $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$. Once sprayed as a suspension on the leaf surface, water evaporates to leave behind a protective particle film. To obtain the desired results on plant tissue, an effective particle film must have certain characteristics. In particular, the mineral particle must have a diameter $< 2 \mu\text{m}$, it must be formulated to spread and create a uniform film, it should transmit photosynthetically active radiation but exclude ultraviolet and infrared radiation to some extent, it should not interfere with gas exchange from plant organs, and, of fundamental importance, it must be removable from harvested commodities [11].

Foliar spraying of kaolin on different woody plants improved their growth rate and increased their leaf Chl content, as compared to non-treated plants [12]. Overall, the protective effect of kaolin is due to an increase in reflected light reaching the surface of plant tissues, which prevents overheating, thus significantly reducing damage by severe sunburn [12]. Moreover, kaolin should be applied before high temperatures are experienced and it must be reapplied to protect new growth or after a heavy rain [11].

The use of protective compounds against sunburn could prove to be an essential and low-cost tool to improve the yield and quality of crops subjected to long periods of extreme solar radiation and high temperatures. Therefore, the objective of this study was to evaluate the efficacy of the protective product Archer[®] Eclipse, created by the company Atlántica Agrícola S.A., against experimentally induced sunburn conditions. The species used was the cucumber (*Cucumis sativus* L.), which is a sensitive species that is usually grown in the summer period and is, therefore, subjected to conditions of high solar radiation and high temperatures during its development.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Cucumber plants (*C. sativus* L. cv. Prior) were used for the experiment. The seeds were germinated and grown for 35 days in a seedbed. Subsequently, the seedlings were transferred to a culture chamber under controlled conditions with a relative humidity of 60–80% (day/night), temperature of 25 °C/15 °C (day/night), and a 16 h/8 h photoperiod with a PPFD (photosynthetic photon-flux density) of $350 \mu\text{mol}^{-2} \text{s}^{-1}$ (measured with an SB quantum 190 sensor, LI-COR Inc., Lincoln, NE, USA). Under these conditions, the plants

were grown in a tray with cells (cell size, 3 cm × 3 cm × 10 cm) filled with a peat:perlite mixture. Fertigation consisted in a complete Hoagland-type nutrient solution, with minor modifications for cucumber cultivation, consisting of: 4 mM KNO₃, 2 mM Ca(NO₃)₂, 2 mM MgSO₄, 1 mM KH₂PO₄, 1 mM NaH₂PO₄, 2 μM MnCl₂, 1 μM ZnSO₄, 0.25 μM CuSO₄, 0.1 μM Na₂MoO₄, 125 μM Fe-EDDHA, and 50 μM H₃BO₃, with a pH of 5.8, applied when needed, according to the requirements of the plants. The plants were watered with 100 mL of water each, when needed.

2.2. Description of the Treatments and Experimental Design

After 35 days, the experiment began with the implementation of the specific condition to generate “sunburn”. This condition consisted of illuminating the plants with high-pressure sodium vapor lamps (SONLIGHT HPS-TS 600W). These lamps were placed at a height of about 60–90 cm from the plant to obtain a level of illumination (PPFD) higher than 1200 μmol m⁻² s⁻¹. These conditions were set daily for a period of 5 h, starting at 12:00 p.m. and ending at 5:00 p.m. The plants were separated into two groups. One group was sprayed with a homogenous layer of Archer[®] Eclipse (2%, v/v) via a foliar spraying technique just one time, and each plant in this group was treated with an amount of 50 mL of spraying solution once the high light and temperature conditions had started. The other group, which was considered the control group, was not sprayed. The experimental design included a complete randomised block with nine plants per treatment arranged in individual cells on trays, with the treatments being randomly distributed in the culture chamber. Archer[®] Eclipse was formulated with calcium (Ca) and zinc (Zn) salts, as well as vegetal extract coming from co-products from the corn processing industry (corn steep liquor, CSL, as a nitrogen source). CSL is a viscous liquid mixture consisting entirely of the water-soluble components of corn steeped in water. The steeping process starts with soaking corn grain in open tanks at 45 to 52 °C for 40 to 48 h. Sulphur dioxide (SO₂) is added to prevent fungi growth and to aid in solubilising the material. Initial concentrations of SO₂ are between 0.1 and 0.2% (pH 3.8 to 4.5) and decrease to 0.05% and 0.01% after 5 and 10 h, respectively. Active fermentation occurs in the steep water, and lactic acid bacterial populations increase as SO₂ concentrations decrease. This separates the starch from the gluten, solubilises and breaks down proteins, and softens the corn to facilitate grinding. The amino acid and peptide rich steep liquor is collected and concentrated. This vegetal extract contains (μg g⁻¹): 4-aminobutyrate (33.20), alanine (311.58), asparagine (70.26), isoleucine (48.43), leucine (103.24), valine (76.99), 3-phenyllactate (46.94), 4-hydroxyphenylacetate (100.16), formate (2.67), lactate (2487.15), indoleacetic acid (7.48), choline (28.96), and trigonelline (3.15). This analysis was performed via nuclear magnetic resonance spectroscopy (NMR): Ascend 500 MHz AVANCE III HD H-NMR (Bremen, Germany). The resulting spectra were evaluated with the programme ‘Chenomx NMR Suite’, version 8.3, and a LC/MSD Trap, version 3.2 (Bruker Daltonik GmbH, Bremen, Germany).

2.3. Plant Sampling

At the end of the experiment, 15 days after the application of sunburn-inducing conditions (das) (when the appearance of sunburn symptoms on leaves was clearly detected), subsamples of plant material were harvested just after daily sunburn treatment was finished. These subsamples were then washed with Milli-Q water and dried on filter paper to obtain fresh weight (FW). Other subsamples were frozen at −40 °C and used for the biochemical analysis. All the plants were harvested and dried in a forced-air oven to determine the dry weight (DW).

2.4. Leaf Temperature and Thermographic Images

Both leaf temperature and thermographic images were taken directly using the HTi Thermal Imaging Camera, model HT-19. The data reported are from the last day of the experiment [13]. Leaf temperature was measured in one leaf of every plant (9 plants per treatment) and randomly in 6 more leaves to increase sample size.

2.5. Gas Exchange Measurements

Measurements were recorded using a LICOR 6800 Portable Photosynthesis System infrared gas analyser (IRGA: LI-COR Inc. Lincoln, NE, USA). Intermediate leaves were placed in the measuring cuvettes under optimal growth conditions. Prior to use, the instrument was warmed for 30 min and calibrated. Measurements used standard optimum cuvette conditions at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) and $400 \mu\text{mol mol}^{-1}$ of CO_2 concentration, and ambient leaf temperature and relative humidity. Net photosynthetic rate, transpiration rate, and stomatal resistance were recorded simultaneously. The data were stored in the LICOR device and analysed using the “Photosyn Assistant” software. Instantaneous water use efficiency (WUE) was calculated by dividing the net photosynthetic rate (A_{CO_2}) by the corresponding transpiration rate (E_{leaf}). The data reported are from the last day of the experiment [14].

2.6. Chl *a* Fluorescence Analysis

Plants were adapted to 30 min of darkness before taking the measurements, using a special leaf clip placed on each leaf. The kinetics of Chl *a* fluorescence were determined using the Handy PEA Chlorophyll Fluorimeter (Hansatech Ltd., King’s Lynn, Norfolk, UK); OJIP phases were induced by red light (650 nm), with a light intensity of $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. OJIP fluorescence phases were analysed using the JIP test [15]. Measurements were performed on fully developed leaves at the mid-plant position of nine plants per treatment. The following parameters obtained from the JIP test were used to study energy fluxes and photosynthetic activity: initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_0$), performance index (PI_{ABS}), the ratio of active reaction centres (RC) (RC/ABS), and the Ψ_{E_0} value, indicating electron output primarily from photosystem II (PSII) [15].

2.7. Concentration of Chls and Carotenoids

The concentrations of Chls and carotenoids were analysed according to [16] with some modifications. Plant material, 0.1 g, was ground with 1 mL of methanol. Subsequently, it was centrifuged for 5 min at $5000 \times g$. The absorbance was measured at three different wavelengths: 666 nm, 653 nm, and 470 nm, and the following calculations were performed based on the following equations:

$$\text{Chl } a \text{ (Chl } a) = 15.65 \times A_{666\text{nm}} - 7.34 \times A_{653\text{nm}} \quad (1)$$

$$\text{Chl } b \text{ (Chl } b) = 27.05 \times A_{653\text{nm}} - 11.21 \times A_{666\text{nm}} \quad (2)$$

$$\text{Carotenoids} = (1000 \times A_{470\text{nm}} - 2.86 \times \text{Chl } a - 129.2 \times \text{Chl } b) / 221 \quad (3)$$

2.8. Electrolyte Leakage

Cell membrane stability was determined by performing the electrical conductivity (EC) test [17]. For this purpose, 0.3 g of fresh plant material was weighed, cut into pieces, lightly washed with deionised water, and placed in a test tube, after which 30 mL of deionised water was added. The tubes were shaken in a vortex for 1 min. The initial conductivity (EC1) was measured using a conductivity meter (Cond 8; XS Instruments, Carpi, Italy). Subsequently, the tubes were incubated in a water bath at a temperature of 100°C for 20 min to extract the released electrolytes and they were allowed to cool to room temperature. Next, the final conductivity (EC2) was measured. The percentage of electrolyte loss was calculated using the following formula: $(\text{EC1}/\text{EC2}) \times 100$.

2.9. Determination of the Concentration of Oxidative Indicators (Malondialdehyde (MDA), H_2O_2 , and O_2^-)

MDA concentration was determined in accord with [18]. Fresh material was extracted with TBA + TCA and, after extraction, absorbance was recorded at 532 nm and 600 nm to correct for turbidity. The H_2O_2 concentration was measured colorimetrically accord-

ing to [19], based on the reaction with KI and reading absorbance at 350 nm. For O_2^- determination, the method described by [20] was followed. The method was based on the reaction with hydroxylamine, sulfanilic acid, and α -1-naphthylamine. Colour intensity was measured at 530 nm.

2.10. Determination of Total Phenol Concentration

Total phenols from plant tissue were extracted with methanol. The content was quantified at an absorbance of 765 nm using the Folin–Ciocalteu reagent [21]. The phenol concentration was obtained using a caffeic acid standard curve.

2.11. Statistical Analysis

The results were statistically evaluated with an analysis of variance (one-way ANOVA), with a 95% confidence interval. All the biochemical analyses were repeated three times and their average was considered one experimental unit. All physiological measurements were taken from one leaf per plant. Thus, the average was obtained from nine experimental units ($n = 9$). Leaf temperature, however, was measured on 15 single leaves per treatment ($n = 15$). Differences between the means of the two treatments were compared using Fisher's least significant difference (LSD) test at a 95% probability level. Significance levels were expressed as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and NS not significant. The experiment was replicated twice and no significant differences were found between the two replications. Data were from the first experiment.

3. Results

3.1. Visual Symptoms and Biomass

High light treatment ($1.200 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 5 h per day caused sunburn symptoms in the leaves of both treated and non-treated plants. Thus, cucumber plants from both treatments showed leaf margin chlorosis and chlorotic spots. However, these symptoms were much more severe in the plants that did not receive the sunblock treatment. Indeed, Archer® Eclipse plants showed leaves that were greener than those from non-treated plants (Figure 1). In addition, the average shoot biomass per plant was significantly higher in plants treated with Archer® Eclipse when compared to the control plants (Figure 2).

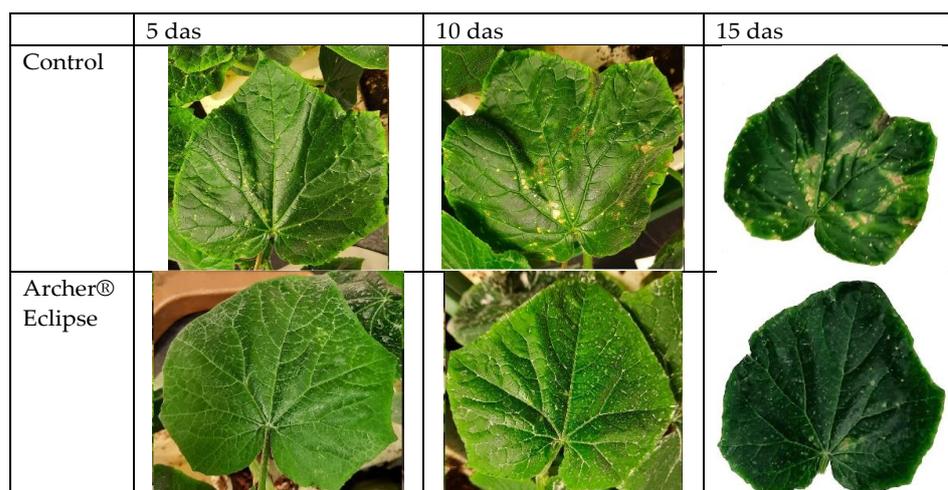


Figure 1. Appearance of leaves in the different treatments, in sampling days, after the application of sunburn-inducing conditions.

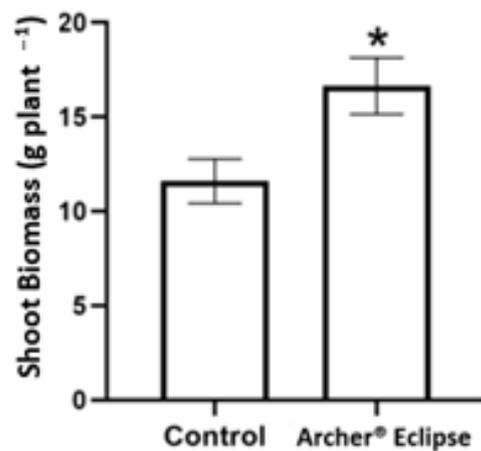


Figure 2. Average shoot biomass of control plants and plants treated with Archer® Eclipse. Values are means ± standard error (n = 9). Differences between means were compared with Fisher’s least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.05$ (*).

3.2. Leaf Temperature

The thermographic study showed that, before the application of high light intensity (time 0 h), the recorded temperature was similar in the plants from both treatments (17–18 °C). When light intensity increased, there was an increase in cucumber plant leaf temperature in the treated and non-treated plants. However, plants treated with Archer® Eclipse showed a lower average temperature in comparison with control plants. Indeed, in the treated plants, the temperature was, on average, 3 °C lower at 2.5 h, and 3.5 °C lower at 5 h, as compared to the control plants (Figure 3). The maximum leaf temperature reached values of 47 °C in the non-treated plants at 5 h after the application of high light intensity had started.

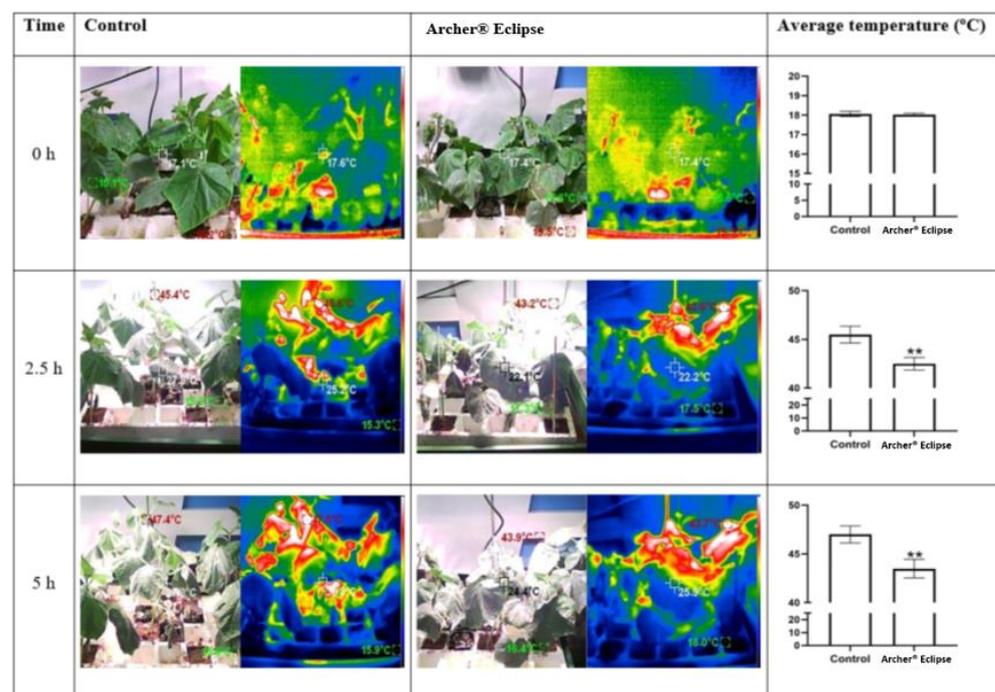


Figure 3. Thermographic images and average temperature at 0 h, 2.5 h, and 5 h from the beginning of the application of sunburn conditions. Values are means ± standard error (n = 15). Differences between means were compared with Fisher’s least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.01$ (**).

3.3. Photosynthetic Parameters

The leaf gas exchange parameters showed that the Archer[®] Eclipse treatment reduced stomatal conductance (g_s) and g_{leaf} values in comparison with the control plants. However, no differences regarding A nor C_i were observed between plants from either treatment. The WUE was higher in plants treated with Archer[®] Eclipse (Table 1). Considering the Chl a fluorescence parameters, Archer[®] Eclipse application enhanced the values of all analysed parameters in comparison with the values of the control plants (Table 2). Furthermore, the concentration of total Chl and the Chl a/b ratio was higher in plants treated with Archer[®] Eclipse, although these plants registered lower carotenoid concentrations, as compared to the control plants (Table 3).

Table 1. Gas exchange parameters of control plants and plants treated with Archer[®] Eclipse.

	G_s ($\text{mol m}^{-2} \text{s}^{-1}$)	g_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol mol}^{-1}$)	A_{CO_2} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol mol}^{-1}$)
Control	0.016 ± 0.002	0.56 ± 0.07	330.16 ± 62.52	0.61 ± 0.12	1.09 ± 0.09
Treatment	0.010 ± 0.004	0.33 ± 0.11	312.99 ± 47.32	0.67 ± 0.17	2.05 ± 0.24
<i>p</i> -value	*	*	NS	NS	**

Values are means \pm standard error ($n = 9$) and differences between means were compared with Fisher's least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.05$ (*) and $p < 0.01$ (**). Leaves were measured 15 days after sunburn conditions with a Portable Photosynthesis System infrared gas analyser.

Table 2. Chl a fluorescence parameters of control plants and plants treated with Archer[®] Eclipse.

	Fv/Fm	PI _{ABS}	RC/ABS	Ψ_{Eo}
Control	0.34 ± 0.11	2.52 ± 1.20	0.38 ± 0.22	0.47 ± 0.08
Treatment	0.83 ± 0.01	10.23 ± 1.09	1.00 ± 0.04	0.67 ± 0.01
<i>p</i> -value	***	***	***	***

Values are means \pm standard error ($n = 9$) and differences between means were compared with Fisher's least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.001$ (***). Measured 15 days after sunburn conditions with a Chlorophyll Fluorimeter.

Table 3. Total Chls and carotenoid concentrations in control plants and plants treated with Archer[®] Eclipse.

	Total Chls (mg g^{-1} FW)	Chl a/b Ratio (-)	Carotenoids (mg g^{-1} PF)
Control	0.373 ± 0.007	1.40 ± 0.03	0.169 ± 0.003
Treatment	0.454 ± 0.008	1.78 ± 0.01	0.159 ± 0.002
<i>p</i> -value	***	***	**

Values are means \pm standard error ($n = 9$) and differences between means were compared with Fisher's least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.01$ (**) and $p < 0.001$ (***). Leaves were measured 15 days after sunburn conditions with a spectrophotometer.

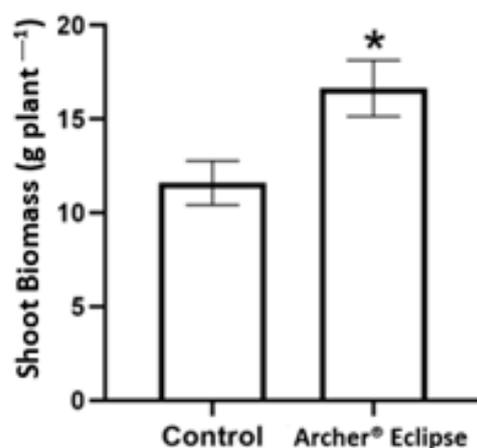
3.4. Oxidative Stress Indicators and Total Phenol Concentration

Plants treated with Archer[®] Eclipse showed significantly lower EC percentage values, MDA, O_2^- , and H_2O_2 concentrations, as compared to the control plants (Table 4). In addition, cucumber plants treated with Archer[®] Eclipse showed lower total phenol concentrations than the control plants (Figure 4).

Table 4. EC percentage and MDA, O_2^- , and H_2O_2 concentrations in control plants and plants treated with Archer[®] Eclipse.

	EC (%)	MDA ($\mu\text{M g}^{-1}$ FW)	O_2^- ($\mu\text{g g}^{-1}$ FW)	H_2O_2 ($\mu\text{g g}^{-1}$ FW)
Control	31.59 \pm 0.46	19.46 \pm 0.92	31.44 \pm 0.27	53.83 \pm 11.11
Treatment	17.26 \pm 0.57	5.61 \pm 0.38	16.27 \pm 0.44	10.97 \pm 4.35
<i>p</i> -value	***	***	***	***

Values are means \pm standard error ($n = 9$) and differences between means were compared with Fisher's least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.001$ (***). Leaves were measured 15 days after sunburn conditions with different chemical procedures.

**Figure 4.** Total phenol concentrations in control plants and plants treated with Archer[®] Eclipse. Values are means \pm standard error ($n = 9$) and differences between means were compared with Fisher's least significant difference test (LSD; $p = 0.05$). The levels of significance were represented by $p < 0.05$ (*).

4. Discussion

Agronomic crops, such as the cucumber, whose growing period in the field occurs in the summer season under stressful conditions such as high degrees of light and temperatures, usually suffer sunburn symptoms that appear in the vegetative parts of the plants. The appearance of these symptoms on leaves generally causes a reduction in plant growth [1,22]. Indeed, biomass reductions under these stressful conditions have been well characterised in various plant types and organs [23,24]. In this experiment, it was reported that cucumber plants are very sensitive to simultaneous excess amounts of light and temperature, as the plants exposed to these conditions had damage in their leaves (Figure 1). However, the plants subjected to sunburn-inducing conditions and treated with Archer[®] Eclipse had no visual damage and presented the greatest shoot growth, as evidenced by the 43% increase in biomass when compared with the non-treated plants. Hence, these results clearly prove the protective effect of Archer[®] Eclipse against high amounts of light and temperature stress. Another piece of evidence of the protective effect under sunburn conditions was observed in the leaf Chl concentration. The plants treated with Archer[®] Eclipse had a higher total Chl concentration than the non-treated plants. The Chl content in leaves is one of the most important physiological characteristics that is altered under this type of stress, and it is considered one of the most important indicators of plants suffering sunburn [25].

The data from the thermographic images and leaf temperature measurements suggest that the application of Archer[®] Eclipse was effective in reducing leaf temperature under sunburn conditions, and this protective effect was maintained over time, thus avoiding leaf damage and favouring plant growth. One of the possible mechanisms of action of Archer[®] Eclipse is the reflection of a portion of the direct incident light that reaches the surface of plant tissues, preventing overheating and leaf damage. Cucumber plants are sensitive to

temperature, and sunburn symptoms normally occur in plants subjected to temperatures above 35 °C [26]. Therefore, any agronomical techniques that are able to reduce the incident light under conditions of high environmental light could favour the growth of the plants. Several studies have also reported that some products formulated with kaolin and calcium sulphate are able to reflect a portion of the direct light radiation that reaches the surface of the plant tissue, preventing overheating and thereby sunburn symptoms [5,12]. In addition, Archer[®] Eclipse application could have reduced the quantity of UVB and UVC reaching the leaf due to the presence of indoleacetic acid (AIA) from the vegetal extracts that the product has. The authors in [27] report that AIA is a natural protector against these types of radiation, which damage DNA molecules by instigating dimerisation and ionisation of pyrimidines, resulting in disturbance of protein synthesis and disrupting of their structures [28,29]. Despite this temperature and light reduction in the leaves, Archer[®] Eclipse application had no effect on gas exchange regulation mechanisms, as A_{CO_2} and g_s were close to zero for both treated and non-treated plants. The daily stress period could have drastically decreased the A_{CO_2} and g_s , as cucumber plants are a thermophilic crop that grows optimally at 25 °C [30], but temperatures above 35–50 °C can lead to leaf damage and even dead tissue [31]. Under these conditions, it has been reported that both thermo-tolerant and thermo-sensitive cucumber species show decreased leaf gas exchange parameters, but only thermo-tolerant cucumber species restore functions to a relatively healthy level after high-temperature exposure [32]. Nevertheless, A_{CO_2} and g_s reduction in treated plants could be due to an adaptation mechanism to high light and temperature favoured by Archer[®] Eclipse's application, since leaf damage was not observed and F_v/F_m , PIABS, RC/ABS, and Ψ_{EO} values were higher than in non-treated plants [15].

Non-treated plants, under high light and temperature conditions, increased their antioxidant response relative to plants treated with Archer[®] Eclipse, as suggested by the data from the Chl *a/b* ratio and the concentrations of carotenoids and phenols in the leaves. This could all suggest that non-treated plants suffered a greater stress intensity than the Archer[®] Eclipse plants. The Chl *a/b* ratio, an indicator of environmental stress [25], was higher in treated than in non-treated plants, confirming that the treated plants suffered less stress. A decrease in this ratio indicates that the plants suffered a strong degree of oxidative stress and activated their antioxidant system to reduce ROS and turn part of Chl *a* into Chl *b*, as this is an O₂ consumption process which removes the aforementioned from the chloroplast. Carotenoids are another set of pigments related to light in plants. Non-treated plants had higher leaf carotenoid concentrations than the plants treated with Archer Eclipse. Changes in leaf carotenoid concentrations could be triggered by ROS accumulation [1,33]. Some of the most important antioxidant compounds in plants are phenols. These compounds appear to be essential as physiological mechanisms for protection against sunburn, as they are capable of absorbing excess light radiation, avoiding or reducing the photo-oxidation process, and directly detoxifying ROS [34]. Non-treated plants had a higher leaf phenol concentration than treated plants, indicating once again that the non-treated plants strongly activated their antioxidant system to cope with high light and temperature conditions. Therefore, non-treated plants, when compared with treated Archer[®] Eclipse plants, suffered from more intense stress, which led to an increase in the activity of antioxidant systems (lowered Chl *a/b* ratio and increased carotenoids and phenols). However, this was not enough to avoid oxidative damage, as indicated by high values of EC and MDA. One of the main effects of the massive presence of ROS in cells is the deterioration of the cell membranes, which leads to an increase in permeability and therefore the loss of electrolytes. The EC percentage and the MDA concentration are two parameters that are indicative of membrane degradation and lipid peroxidation, and the increase in their values suggests the excessive presence of ROS [35]. Thus, Archer[®] Eclipse foliar application avoided ROS formation and loss of cell permeability by reflecting a portion of the direct light that reaches the surface of the plant tissues. In addition, it can also not be ruled out that ROS scavenging could have been increased by the Archer[®] Eclipse product due to the direct action of sugar

and amino acids contained in the botanical extract of its formulation. Amino acids and sugars are powerful antioxidants that are able to cope with abiotic stresses [30,36].

In a future experiment, it will be necessary to address how to optimise Archer® Eclipse use, mainly by increasing knowledge on the plant's response to its application. More specifically, complementary studies should be carried out to analyse the antioxidant system of the plants in detail, collect more information about crops that could benefit from this technology, and determine the best time and frequency of application. Moreover, studies examining the interactions of Archer® Eclipse application with other agronomic practices, such as the use of cover crops and deficit irrigation strategies, are needed to improve the effectiveness of Archer® Eclipse under more severe stress conditions.

5. Conclusions

This study confirms the efficiency of Archer® Eclipse in protecting the cucumber plant from sunburn-inducing conditions. Furthermore, due to its composition, a protective effect of the pit-dye mechanism is evident. Having said that, more studies are needed in order to fully understand the protective role of the organic compounds in the Archer® Eclipse vegetal extract and their synergies with each other.

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