

## Article

# Salicylic Acid Foliar Application Increases Crop Yield and Quality Parameters of Green Pepper Fruit during Postharvest Storage

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**Abstract:** The main aim of this study was to evaluate the effect of salicylic acid (SA) as a preharvest treatment on crop yield throughout the crop cycle of green pepper fruit as well as on its quality parameters, including functional quality, at harvest and during 21 days of storage at 7 °C. Thus, ‘Herminio’ pepper plants were treated with SA at 0.5, 1 and 5 mM, and higher crop yield (kg per plant, number of fruits per plant and average fruit weight) and quality parameters (firmness, green color and total acidity) at harvest were obtained with the 0.5 mM dose, as well as greater phenolic compounds content and total antioxidant activity. These quality traits and functional quality were also maintained at higher levels for this treatment than in controls during postharvest storage, leading to a delay of fruit quality losses. In addition, the decay incidence for 0.5 mM SA-treated pepper fruits reached a ca. value of 2% at the end of the storage, which was lower than untreated fruits (16.6%). These results suggest that preharvest application of SA at low doses tested on pepper plants could be a useful tool to increase crop yield and fruit quality parameters at harvest and maintain them during storage, delaying quality losses and decay incidence.

**Keywords:** average weight; decay incidence; firmness; SA; total phenolics



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## 1. Introduction

Crops and their supplies require substantial increments for servicing the gap between production and demand [1]. In the last few years, the necessity of improving crop yield has been much more emergent due to the expanding population [2]. Research has been performed in recent years regarding the use of preharvest treatments using naturally occurring plant compounds to increase crop yield and fruit quality at harvest and to maintain it during storage, due to consumers’ concerns and legal restrictions concerning postharvest chemical treatments [3]. The use of plant growth regulators [4] has been recently introduced to improve the quality of vegetable products. Salicylic acid (SA) is a water-soluble secondary metabolite and a phenolic compound produced by the plant organism [5]. This ubiquitous phytohormone is essential for plant growth, development and stress resistance [6]. SA acts as a signaling agent in plants which promotes tolerance against several biotic and abiotic stresses [7] and regulates many physiological and metabolic processes [8], such as proline metabolism, photosynthesis, transpiration, ion uptake and transportation [2,9]. Moreover, SA has been shown to intervene with the ethylene, abscisic acid and cytokinins roles in plants [10]. Therefore, SA has recently been proposed to be a new kind of plant growth regulator.

Pepper fruit (*Capsicum annuum* L.) is a vegetable of great economic importance worldwide, and it is highly appreciated in the market for its organoleptic qualities [11]. Further, consumers purchasing decisions commonly focused on the color, size and firmness, among other quality traits [12]. In particular, the freshness of the pepper is an important quality

attribute to consumers [10]. In addition, this vegetable is a good source of antioxidants, flavonoids, phenolic acids and carotenoids [13,14]. Green peppers are harvested before they ripen completely and, recently, the phenological stage and harvest date have been defined as two key factors that significantly influence their bioactive compounds content. The later phenological stages and harvest dates which have been studied have revealed that there could be a greater benefit to health [15].

Beyond the upsides of this crop, as a subtropical crop, pepper fruit is susceptible to chilling injury (CI) at temperatures  $< 7$  °C, leading to superficial pitting, watery stains and seed and calyx browning. The major postharvest problem when pepper fruits are stored at non-chilling temperatures is excessive softening that may cause shrinkage, drying and pathological disorders, such as gray mold caused by *Botrytis cinerea*, which reduce the product quality and acceptability. In fact, handlers and consumers focus a lot of importance on the retention of quality attributes, such as fruit green color, freshness and firmness, during postharvest handling and storage [10]. In addition, other factors such as absence of defects, diseases and shelf-life are also considered [16]. In this sense, the storage or marketable life of harvested green pepper fruits can be extended by various treatments applied to them postharvest. As a postharvest treatment in pepper fruit, SA could delay the softening process, maintaining fruit quality and retaining the nutritional quality of sweet peppers during storage at 25 °C and at 10 °C [10]. Furthermore, a single SA treatment or a combined SA treatment with trisodium phosphate (TSP) was found to enhance cold tolerance through the effects on antioxidant metabolism or inhibiting the CI-induced membrane damage [6,17].

On the other hand, control of postharvest diseases is commonly linked to the use of synthetic fungicides, which is being increasingly legislated in order to avoid their potential risk for consumer health and to promote the use of eco-friendly strategies [18]. Preharvest applications of SA have stimulated the disease resistance against fungal infection of pepper fruits [19–22]; accordingly, it also reduced the decay incidence and postharvest losses during storage. In the scientific literature, studies focusing on the action of SA preharvest application on salinity stress oxidative damage of sweet pepper plants are also incipient [2,23,24]. Nevertheless, few studies have investigated the effect of SA preharvest treatment on increasing crop yield and quality of pepper fruit [25,26]. Both studies are focusing on the effect at one harvest date along the crop cycle [25] and on the quality of red sweet pepper cultivars [26]; however, no information is available about these effects on green pepper fruit. Pepper fruit, as non-climacteric fruit, show a low profile and a gradual decline in its respiration pattern through the ripening process [27]. This physiological behavior has a great importance in the postharvest biology and technology of green pepper fruit. As far as we know, there is no scientific literature regarding the effect of SA preharvest treatment on crop yield throughout the developmental and growth cycle of green pepper fruit as well as on its quality parameters, including functional quality, at harvest and during 21 days of storage at 7 °C, which is the main aim of this research.

## 2. Materials and Methods

### 2.1. Plant Material, Treatments and Growth Conditions

For this experiment, pepper plants (*Capsicum annuum* L.) of ‘Herminio’ cultivar were planted on January 2020 in a commercial plot growing under plastic-roofed greenhouse located in El Raal (Murcia, Spain). The experiment was conducted from February to July 2020 (Table 1). Thus, 180 pepper plants were selected and distributed in randomized complete block design with twelve replicates or blocks in total. Each treatment was performed in three blocks ( $n = 3$ ) of 15 plants (45 plants per block and treatment). Treatments were a control (plants treated with distilled water) and salicylic acid (SA) (plants treated with the reagent purchased from Sigma, Sigma-Aldrich, Madrid, Spain; CAS Number: 69-72-7) at three concentrations: 0.5, 1 and 5 mM. These doses were chosen according to previous experiments in which 0.5, 1.0 and 2.0 mM doses of SA were applied to sweet cherry and plum trees [28,29] and higher doses (SA 5 mM) have recently been applied on

pomegranate trees [30]. Seven exogenous applications by foliar spray throughout the crop cycle were performed (Table 1), the first treatment was applied before the beginning of the flowering stage. The equidistance among application dates was ca. 21 days due to a staggered flowering cycle, except for the last application that was performed close to the last commercial harvest, being chosen based on the crop cycle duration of this pepper cultivar. Crop management was performed according to the usual crop program designed by the company for the short-term crop cycle of ‘Lamuyo’ pepper type, in which rockwood was used as the soil substrate and drip irrigation and optimal nutrient levels were applied. The soil texture was sandy loam with a pH of 7.50.

**Table 1.** Application dates of treatments (control and SA at 0.5, 1 and 5 mM) throughout the developmental and growth cycle of ‘Herminio’ green pepper fruit.

Treatments	T1	T2	T3	T4	T5	T6	T7
Dates	24 February	17 March	6 April	29 April	19 May	9 June	12 July

Pepper fruits were harvested at the commercial harvest stage when green pepper had reached the phenological stage suitable for its consumption. A total of 10 harvest dates throughout the developmental and growth cycle were performed according to a staggered production and the commercial criteria of harvesting green pepper fruit established by the company. The harvest dates started from April until July: 6 April, 20 April, 4 May, 14 May, 26 May, 4 June, 16 June, 26 June, 6 July and 17 July. The mean temperature for each month was recorded: April (14.58 °C), May (20.06 °C), June (23.33 °C) and July (25.98 °C), using a station close to the experimental greenhouses (38°2′2.64″ North, 1°1′18.9″ West).

## 2.2. Crop Yield

Parameters related to crop yield were evaluated for each harvest date along the crop cycle and blocks designed per treatment. Accumulative crop yield was expressed as kg plant<sup>-1</sup> and number of peppers harvested plant<sup>-1</sup>. The average fruit weight (g) was analyzed by weighing all harvested pepper fruits individually.

## 2.3. Experimental Postharvest Storage Design

Pepper fruits harvested on 4 May, this date was chosen according to one previous experiment [15], without visual defects were selected and transferred to the laboratory to carry out a postharvest storage experiment. For each treatment and sampling date, 6 peppers were selected for 3 replicates (18 pepper fruits in total), which were weighted and stored at 7 °C and 85% of relative humidity (RH). Specifically, 72 pepper fruits were stored for each treatment. Pepper fruits were analyzed at harvest (day 0) and at 7, 14 and 21 days of storage. For the experimental postharvest storage, 54 fruits in total were used for the analyses on the three sampling dates (7, 14 and 21 storage days). However, another 18 pepper fruits were stored and weighed to replace rotten pieces of pepper fruit; thus, fruit quality was always analyzed on 18 non-rotten pepper fruits on each sampling date. For each sampling date, weight loss, fruit respiration rate, firmness, color, total soluble solids, titratable acidity, total phenolics content, total antioxidant activity and the incidence of decay were measured. All the analyses were performed on 18 green pepper fruits, this number being representative of each treatment and sampling date.

## 2.4. Quality Parameters of Green Pepper Fruit

Weight loss was measured for each individual lot by recording the pepper fruit weight at harvest (day 0) and at 7, 14 and 21 days of storage. Accumulative weight loss was expressed as a percentage (%) with respect to pepper fruit weight at day 0. Respiration rate was determined following the protocol described by Giménez et al. [31] with slight modifications. Pepper fruits were placed individually in 2 L capacity glass jars, hermetically sealed for 60 min. Thus, CO<sub>2</sub> concentration was measured using a ShimadzuTM GC-14B

(Kyoto, Japan) gas chromatograph equipped with thermal conductivity (TCD). Results were expressed in  $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ .

Firmness was determined individually in each pepper fruit using a TX-XT2i Texturimeter (Stable Microsystems, Godalming, UK). A flat steel plate, mounted on the machine, measured the equatorial fruit diameter and applied a force that achieved a 5% deformation of this diameter. Results were expressed as a force-deformation ratio ( $\text{N mm}^{-1}$ ). Color was measured on three points of the equatorial pepper fruit perimeter by using a Minolta colorimeter (CFRC400, Minolta Camera Co., Kantō, Tokio, Japan) using the CIELab coordinates and was expressed as a  $b^*$  parameter.

Pepper samples of each replicate were combined to obtain a homogeneous sample of juice for each replicate. Total soluble solids (TSS) content was measured in duplicate from the juice obtained from 50 g of pepper fruit, using a digital refractometer (Atago PR-101, Atago Co., Ltd., Tokyo, Japan) at 20 °C and expressed as  $\text{g kg}^{-1}$  of fresh weight (FW). Titratable acidity (TA) was determined in duplicate from the same juice by automatic titration (785 DMP Titrino, Metrohm, Burladingen, Germany) with 0.1 N NaOH up to a pH of 8.10, using 1 mL of diluted juice in 25 mL distilled  $\text{H}_2\text{O}$ , and results were expressed as g malic acid equivalent  $\text{kg}^{-1}$  FW.

### 2.5. Total Phenolics Content and Hydrophilic and Lipophilic Total Antioxidant Activity

All green pepper fruits were cut to remove the peduncle and the seeds at each sampling date and then frozen in liquid  $\text{N}_2$  and maintained at  $-80$  °C until functional analysis. Total phenolic content and total antioxidant activity were measured according to the protocol described in green pepper fruit by Dobón-Suárez et al. [15]. Briefly, 5 g of green pepper fruit were homogenised in 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate. The extracts were centrifugated at  $10,000 \times g$  for 15 min at 4 °C and the upper and lower fractions were used to quantify total lipophilic (L-TAA) and hydrophilic (H-TAA) antioxidant activity, respectively. Total phenolic content was quantified in duplicate on the lower fraction for each extract using the Folin–Ciocalteu reagent, as previously described by García-Pastor et al. [30]. Results for this parameter were expressed as g gallic acid equivalent (GAE)  $\text{kg}^{-1}$  FW. On the other hand, H-TAA and L-TAA were also determined in duplicate in each extract sample, by using the ABTS-peroxidase system, as described by Dobón-Suárez et al. [15]. Results of both parameters, H-TAA and L-TAA, were expressed as g of Trolox Equivalent (TE)  $\text{kg}^{-1}$  FW.

### 2.6. Incidence Decay

Weekly, for each sampling date, the number of decay peppers per treatment stored at 7 °C was recorded. Results were expressed as the percentage (%) of accumulated decay at 21 days of storage with respect to the total number of pepper fruits used in the present experiment (54 pepper fruits in total). In addition, photographs were taken to display the visual aspect of green pepper fruits treated with SA and untreated during 21 days of storage. Photographs of pepper fruits were captured using a digital camera (Nikon D3400) in a light box with white background. The setup conditions of the camera were as follows: light provided by two LEDs of color temperature of 5600 K, flash speed of 1/5 s, ISO-200, focal opening (f) 20, and length 35 mm, according to the described conditions by García-Pastor et al. [32]. The rotten pieces of pepper fruit were assessed, photographed and removed from the experiment.

### 2.7. Statistical Analysis

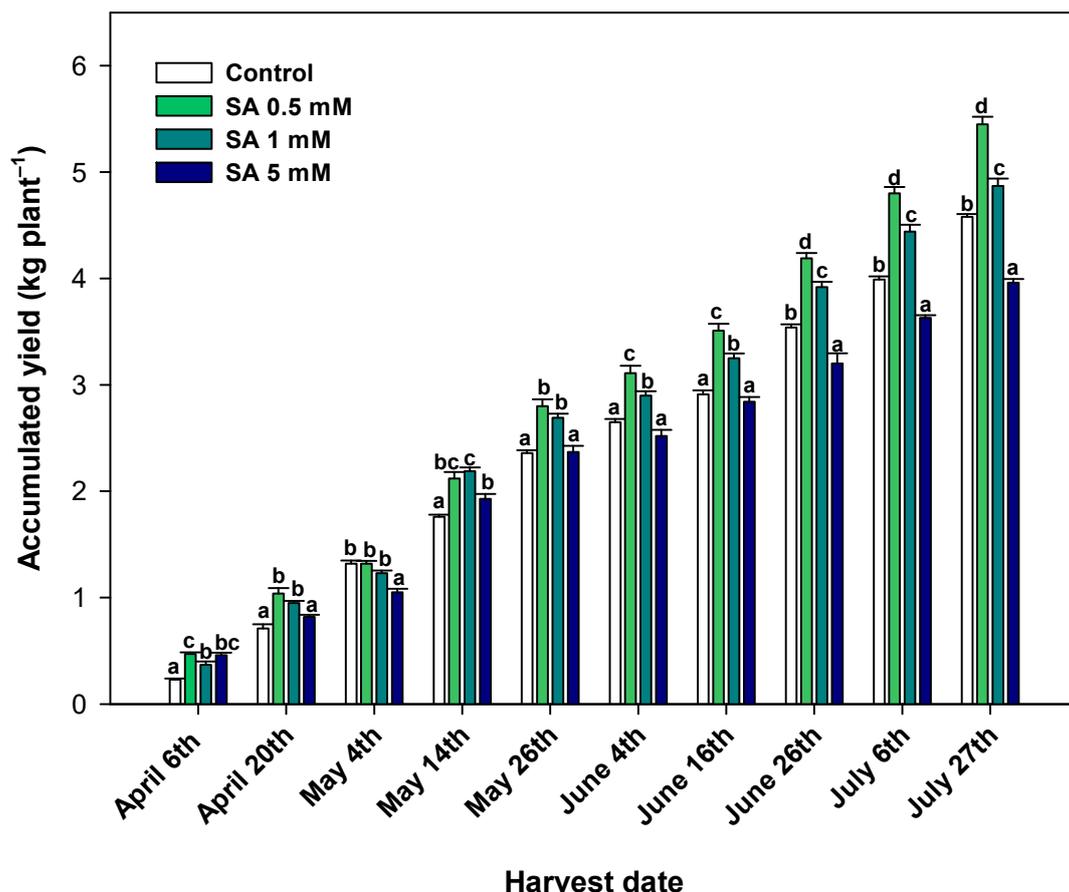
Statistical analysis was carried out in three replicates for all analytical determinations. Results were expressed as the mean  $\pm$  SE. Data were subjected to analysis of variance (ANOVA). The sources of variation were treatments and storage time. Mean comparisons were performed using Tukey's HSD test to determine whether the differences among the treatments or storage time were significant at  $p < 0.05$ . All analyses were performed using

the SPSS software package v.17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA).

### 3. Results

#### 3.1. Effect of SA Preharvest Treatment on Crop Yield

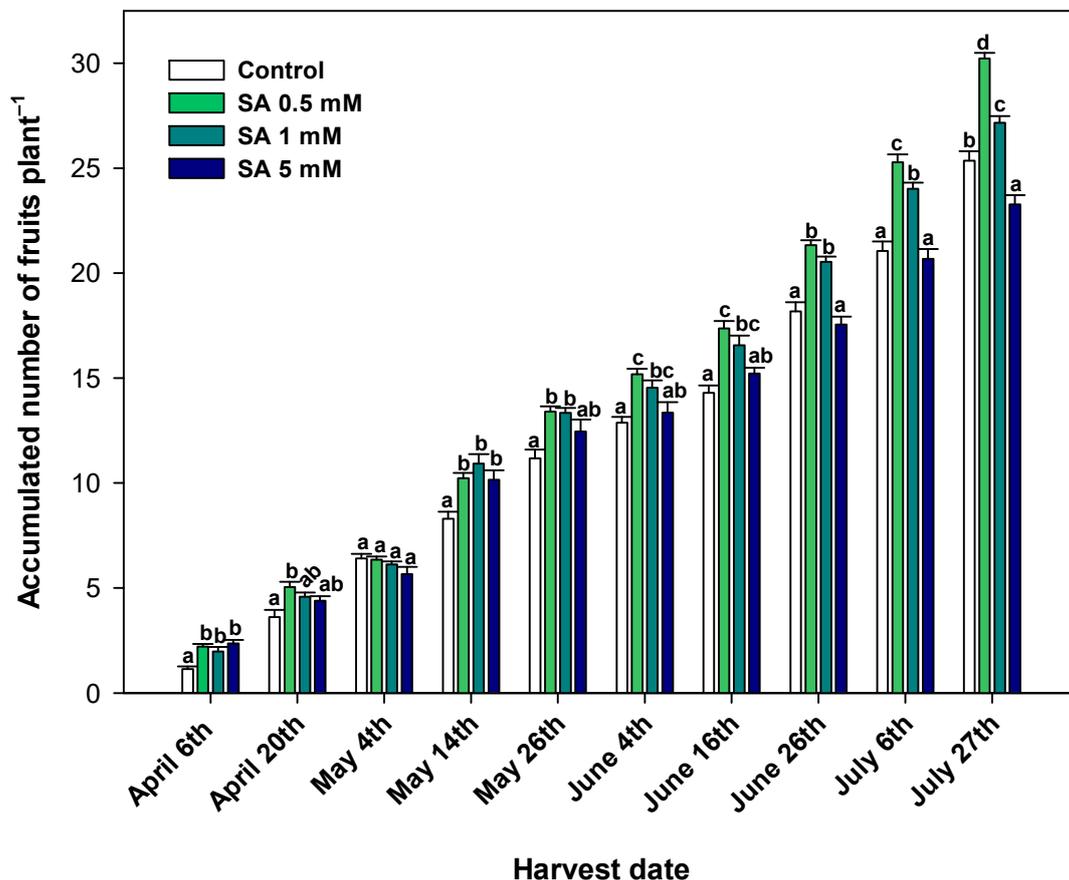
Crop yield, expressed as kg per tree, number of fruits per plant and average fruit weight, is presented in Figures 1–3, respectively. Accumulated yield ( $\text{kg plant}^{-1}$ ) was recorded throughout the developmental and growth cycle of the green pepper fruit, specifically on ten harvest dates from 6 April to 27 July in the 2020 experiment (Figure 1). Results showed that kg of green pepper fruits harvested by plant were significantly higher ( $p < 0.05$ ) for the 0.5 and 1 mM SA-treated plants than in controls, although these increases were not observed in 5 mM SA treated plants. From 6 April to 27 July, SA applied at 0.5 mM was the most effective for increasing accumulated yield, followed by 1 mM SA. In this sense, SA preharvest treatments applied at 0.5 and 1 mM significantly increased ( $p < 0.05$ ) the kg of green pepper fruits harvested per plant, reaching 0.87 and 0.29 kg more than control plants, respectively, at the last harvest date (27 July). In addition, a 2.0- and 1.6-fold increase by 0.5 and 1 mM SA treatments, respectively, was observed at the first harvest date (6 April). However, SA applied at the higher concentration (5 mM) showed a significant decrease in the accumulated yield, leading to 0.62 kg less than untreated plants on 27 July.



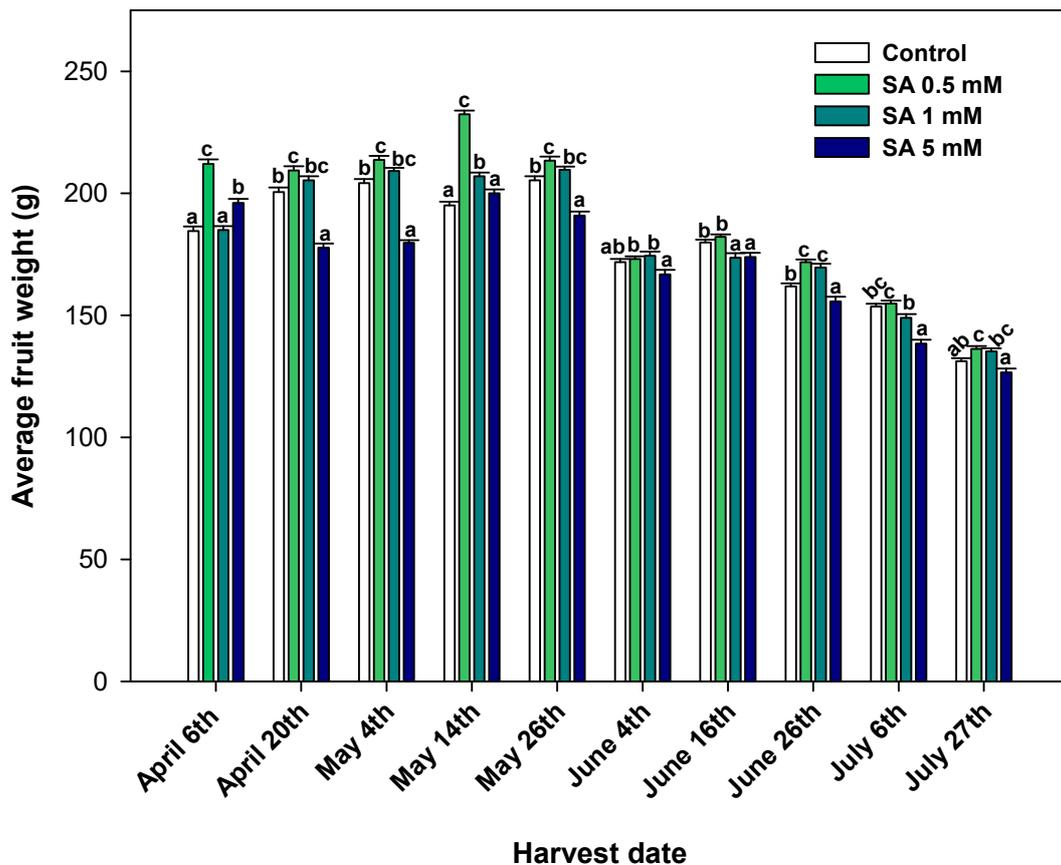
**Figure 1.** Accumulated yield ( $\text{kg plant}^{-1}$ ) throughout the developmental and growth cycle of green pepper fruit, as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean  $\pm$  SE. Different lowercase letters show significant differences among treatments for each harvest date at  $p < 0.05$ .

The number of pepper fruits per plant was also significantly higher ( $p < 0.05$ ) in the 0.5 mM SA-treated plants than in controls throughout the developmental and growth cycle of green pepper fruit. In addition, this increase was also significant ( $p < 0.05$ ) in the 1 mM SA treated plants compared to untreated plants but generally to a lesser extent than the lowest concentration of SA tested (Figure 2). Nevertheless, in the same way that it was observed for accumulated yield (Figure 1), SA treatment applied at 5 mM significantly reduced ( $p < 0.05$ ) the number of pepper fruits harvested per plant at the end of the crop cycle (27 July), although all treatments with SA significantly increased ( $p < 0.05$ ) this parameter on 6 April (Figure 2).

A drastic downward trend was observed for the average weight of green pepper fruit throughout its developmental and growth cycle (Figure 3). The average fruit weight, taking into account data from 6 April to 27 July, was significantly higher ( $p < 0.05$ ) in green pepper fruit from 0.5 mM SA treated plants than from controls, although no significant ( $p \geq 0.05$ ) effect was generally observed with 1 mM SA treatment along the crop cycle (Figure 3). In relation to the other results discussed above, 5 mM SA-treated plants produced green pepper fruits with a significantly lower ( $p < 0.05$ ) average fruit weight than the other treatments in most of the harvest dates studied, except for the first harvest (6 April).



**Figure 2.** Accumulated number of pepper fruits per plant (number of fruits plant<sup>-1</sup>) throughout the developmental and growth cycle of green pepper fruit as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean  $\pm$  SE. Different lowercase letters show significant differences among treatments for each harvest date at  $p < 0.05$ .



**Figure 3.** Average fruit weight (g) throughout the developmental and growth cycle of green pepper fruit as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean  $\pm$  SE. Different lowercase letters show significant differences among treatments for each harvest date at  $p < 0.05$ .

### 3.2. Effect of SA Preharvest Treatment on Weight Loss, Respiration Rate and Physico-Chemical Parameters at Harvest and during Storage

Weight loss increased during postharvest storage in green pepper fruit for all treatments studied, although these increases were significantly reduced ( $p < 0.05$ ) by the SA treatment applied at 0.5 mM after 14 days of storage at 7 °C (Table 2). Thus, peppers treated with SA at the lowest concentration showed the lowest percentage of weight loss at the end of the storage (21 days), although 1 and 5 mM SA treatments did not show significant differences ( $p \geq 0.05$ ) compared to pepper fruit harvested from the control plants. On the other hand, 0.5 mM SA showed the lowest respiration rate at harvest, followed by SA treatments applied at the other concentrations (Table 2). This parameter significantly decreased ( $p < 0.05$ ) from harvest until 21 days of storage at 7 °C in all treatments tested. The effect of SA treatments at 0.5, 1 and 5 mM decreased the respiration rate by 1.33, 1.21 and 1.24-fold compared to the control pepper fruits, respectively, at the end of the storage period, although no significant differences among SA treatments were observed.

**Table 2.** Effects of salicylic acid (SA) preharvest treatments at 0.5, 1 and 5 mM on weight loss (%), respiration rate ( $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) and physico-chemical parameters: firmness ( $\text{N mm}^{-1}$ ), color ( $b^*$ ), total soluble solids (TSS;  $\text{g kg}^{-1}$ ) and total acidity (TA;  $\text{g kg}^{-1}$ ) content of green pepper fruit during 21 days of cold storage at  $7^\circ\text{C}$ <sup>1</sup>.

	Days	Control	SA 0.5 mM	SA 1 mM	SA 5 mM
Weight loss (%)	0	-	-	-	-
	7	2.62 ± 0.16 aA	2.29 ± 0.17 aA	2.42 ± 0.19 aA	2.54 ± 0.17 aA
	14	5.24 ± 0.44 bB	3.90 ± 0.24 aB	4.51 ± 0.25 abB	4.72 ± 0.41 abB
	21	7.50 ± 0.52 bC	5.81 ± 0.35 aC	6.62 ± 0.38 abC	6.70 ± 0.49 abC
Respiration rate ( $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ )	0	86.62 ± 2.91 cC	65.02 ± 1.62 aC	73.51 ± 2.63 bC	74.65 ± 2.86 bC
	7	18.43 ± 0.44 cB	12.73 ± 0.44 aB	14.37 ± 0.43 bB	14.67 ± 0.41 bB
	14	17.34 ± 0.75 bB	11.47 ± 0.72 aAB	12.22 ± 0.72 aAB	12.15 ± 0.67 aA
	21	14.15 ± 0.35 bA	10.63 ± 0.39 aA	11.73 ± 0.59 aA	11.43 ± 0.38 aA
Firmness ( $\text{N mm}^{-1}$ )	0	4.77 ± 0.11 aC	5.84 ± 0.11 cD	5.51 ± 0.14 bcC	5.35 ± 0.11 bC
	7	3.31 ± 0.17 aB	4.65 ± 0.17 bC	3.55 ± 0.19 aB	3.69 ± 0.19 aB
	14	2.94 ± 0.15 aB	3.70 ± 0.13 bB	3.20 ± 0.15 abB	3.03 ± 0.18 aB
	21	1.08 ± 0.18 aA	2.20 ± 0.18 bA	1.84 ± 0.11 bA	1.73 ± 0.12 bA
Color ( $b^*$ )	0	23.57 ± 0.63 aC	21.75 ± 0.69 aC	22.04 ± 0.68 aC	23.43 ± 0.64 aC
	7	20.89 ± 0.51 bB	16.67 ± 0.58 aB	17.22 ± 0.61 aB	18.70 ± 0.63 aB
	14	17.05 ± 0.35 bA	15.34 ± 0.54 aAB	15.70 ± 0.55 abAB	15.68 ± 0.59 abA
TSS ( $\text{g kg}^{-1}$ )	21	16.00 ± 0.38 bA	14.50 ± 0.31 aA	15.00 ± 0.51 abA	15.00 ± 0.51 abA
	0	45.33 ± 1.02 aA	47.16 ± 1.17 aA	46.16 ± 1.42 aA	46.00 ± 1.20 aA
	7	46.66 ± 1.16 aA	49.00 ± 1.73 aA	48.00 ± 1.31 aA	47.66 ± 1.19 aA
	14	47.90 ± 1.42 aA	49.46 ± 1.35 aA	48.66 ± 1.62 aA	48.33 ± 1.85 aA
TA ( $\text{g kg}^{-1}$ )	21	48.30 ± 1.50 aA	50.83 ± 1.16 aA	50.33 ± 1.23 aA	49.90 ± 1.42 aA
	0	1.91 ± 0.07 aB	2.26 ± 0.05 cC	2.17 ± 0.07 bcC	1.96 ± 0.06 abB
	7	1.75 ± 0.04 aB	1.92 ± 0.04 bB	1.90 ± 0.06 abB	1.89 ± 0.07 abB
	14	1.54 ± 0.06 aA	1.87 ± 0.07 bAB	1.81 ± 0.05 bAB	1.75 ± 0.08 abAB
	21	1.35 ± 0.08 aA	1.68 ± 0.06 bA	1.61 ± 0.07 bA	1.52 ± 0.06 abA

<sup>1</sup> Values (mean of three replicates) ± SE followed by different lowercase letters, within the same row, show significant differences ( $p < 0.05$ ) among treatments, according to Tukey's HSD test, for each parameter. Different capital letters in the same column show significant differences among storage days for each treatment at  $p < 0.05$ .

Fruit firmness at harvest was significantly higher ( $p < 0.05$ ) in fruit from SA treated plants than in controls (Table 2). However, SA applied at 0.5 and 1 mM were the most effective treatments for increasing the firmness levels of pepper fruits at harvest, since a 1.20-fold increase was achieved in those SA treated peppers compared to untreated. During postharvest storage, fruit firmness decreased in green pepper fruits from the control and treated plants, maintaining the differences found at harvest. With respect to color, expressed as  $b^*$  parameter, no significant differences ( $p \geq 0.05$ ) were observed among treatments at harvest (Table 2). Nevertheless, after 21 days of storage at  $7^\circ\text{C}$ , all pepper fruits showed important color losses, leading to fruit yellowing, which was significantly delayed ( $p < 0.05$ ) by the 0.5 mM SA treatment.

TSS content did not show significant differences ( $p \geq 0.05$ ) neither among treatments nor during storage days (Table 2). However, TA content was ca.  $2.2 \text{ g kg}^{-1}$  in green pepper fruits treated with SA at 0.5 and 1 mM concentrations (Table 2), being significantly higher ( $p < 0.05$ ) than untreated pepper fruits. During storage, decreased trends in TA were observed for control and SA treated fruit; although significantly higher ( $p < 0.05$ ) levels were maintained in 0.5 and 1 mM SA-treated peppers until the last sampling date.

### 3.3. Effect of SA Preharvest Treatment on Total Phenolics Content and Total Antioxidant Activity at Harvest and during Storage

Total phenolic content as well as hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity were significantly increased ( $p < 0.05$ ) during 21 days of postharvest storage at  $7^\circ\text{C}$  regardless of treatment tested (Table 3). Specifically, total phenolics at harvest were significantly higher ( $p < 0.05$ ) in 0.5 and 1 mM SA-treated green pepper fruits, reaching values of  $0.850 \text{ g kg}^{-1}$  FW, compared to controls (ca.  $0.700 \text{ g kg}^{-1}$  FW). At 21

days of storage, significant differences ( $p < 0.05$ ) on total phenolic content were observed in 0.5 mM SA-treated green pepper fruit compared with control fruits.

**Table 3.** Effects of preharvest salicylic acid (SA) treatments (0, 0.5, 1 and 5 mM) on total phenolics content ( $\text{g kg}^{-1}$ ) and total antioxidant activity (TAA;  $\text{g kg}^{-1}$ ): hydrophilic (H-TAA) and lipophilic (L-TAA) fractions, of green pepper fruit during 21 days of cold storage at  $7^\circ\text{C}$ <sup>1</sup>.

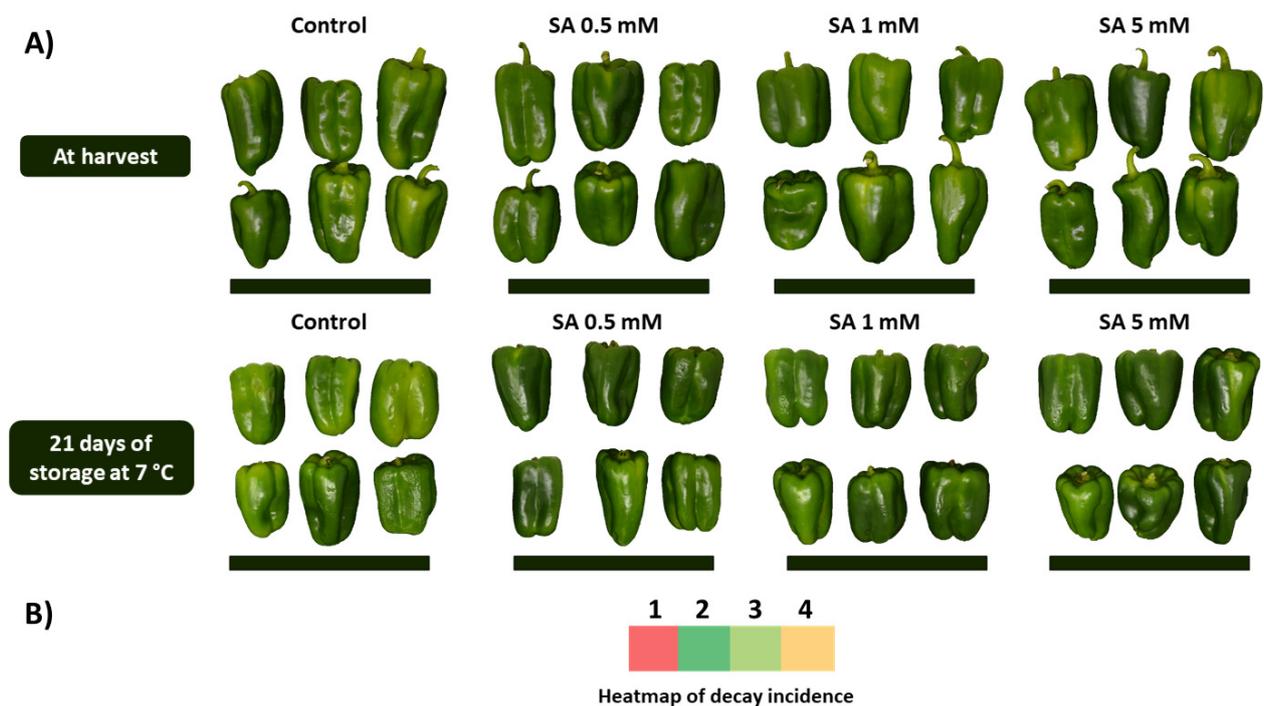
	Days	Control	SA 0.5 mM	SA 1 mM	SA 5 mM
Total phenolic content ( $\text{g kg}^{-1}$ )	0	0.707 ± 0.034 aA	0.854 ± 0.027 bA	0.850 ± 0.036 bA	0.828 ± 0.036 abA
	7	0.800 ± 0.031 aAB	0.928 ± 0.032 bAB	0.900 ± 0.036 abAB	0.914 ± 0.036 abAB
	14	0.870 ± 0.029 aBC	0.987 ± 0.028 bBC	0.952 ± 0.037 abAB	0.950 ± 0.025 abB
	21	0.947 ± 0.026 aC	1.049 ± 0.024 bC	1.017 ± 0.040 abB	0.993 ± 0.037 abB
H-TAA ( $\text{g kg}^{-1}$ )	0	0.750 ± 0.059 aA	1.012 ± 0.049 bA	1.008 ± 0.043 bA	1.004 ± 0.039 bA
	7	1.028 ± 0.053 aB	1.401 ± 0.055 bB	1.287 ± 0.048 bB	1.282 ± 0.044 bB
	14	1.212 ± 0.049 aBC	1.614 ± 0.040 bC	1.522 ± 0.060 bC	1.498 ± 0.054 bC
	21	1.407 ± 0.063 aC	1.866 ± 0.058 cD	1.620 ± 0.036 bC	1.673 ± 0.035 bD
L-TAA ( $\text{g kg}^{-1}$ )	0	0.357 ± 0.022 aA	0.468 ± 0.033 bA	0.381 ± 0.017 abA	0.383 ± 0.020 abA
	7	0.386 ± 0.027 aAB	0.521 ± 0.038 bA	0.395 ± 0.024 aAB	0.392 ± 0.035 abAB
	14	0.430 ± 0.029 aAB	0.647 ± 0.030 bB	0.497 ± 0.041 aBC	0.493 ± 0.043 aAB
	21	0.467 ± 0.030 aB	0.729 ± 0.033 bB	0.539 ± 0.036 aC	0.535 ± 0.050 aB

<sup>1</sup> Values (mean of three replicates) ± SE followed by different lowercase letters, within the same row, show significant differences ( $p < 0.05$ ) among treatments, according to Tukey's HSD test, for each parameter. Different capital letters in the same column show significant differences among storage days for each treatment at  $p < 0.05$ .

H-TAA at harvest was significantly higher ( $p < 0.05$ ) in pepper fruits harvested from SA-treated plants compared to those from untreated plants, although no significant differences ( $p \geq 0.05$ ) were shown among the three concentrations of SA treatments (Table 3). After the increase of H-TAA during storage at  $7^\circ\text{C}$ , 0.5 mM SA was the most effective treatment increasing this functional parameter at 21 days of storage, followed by SA at 1 and 5 mM, while pepper fruits from control plants had the lowest values. Enhanced L-TAA at harvest was only obtained by 0.5 mM SA treatment (Table 3), since green pepper fruits treated with SA at 1 and 5 mM showed similar values of L-TAA than controls. These differences observed for L-TAA levels among treatments were maintained during the 21 days of storage, with SA applied at the lowest concentration the most effective for increasing TAA attributed to compounds presented on the lipophilic fraction.

#### 3.4. Effect of SA Preharvest Treatment on Visual Aspect and Decay Incidence of Green Pepper Fruit during Storage

Both visual aspect and decay incidence of green pepper fruits were recorded at the end of postharvest storage experiment, as can be observed in Figure 4. The greatest valued visual aspect at 21 days of storage at  $7^\circ\text{C}$  in terms of higher green color presence, absence of wrinkling or drying as well as other visual defects, was presented by green pepper fruits treated with SA (Figure 4A). However, those fruits that showed a greater visual aspect of freshness were obtained from 0.5 mM SA-treated pepper plants. Furthermore, this preharvest treatment was the most effective for reducing the decay incidence during 21 days of storage at  $7^\circ\text{C}$ , since the lowest percentage (%) of decay was recorded for this treatment (Figure 4B). Specifically, the decay incidence for 0.5 mM SA-treated pepper fruits reached a ca. value of 2% at the end of the storage, which was lower than untreated fruits (16.6%) and the other pepper fruits treated with SA at 1 and 5 mM (3.7 and 9.3%, respectively).



**Figure 4.** (A) Visual aspect of ‘Herminio’ cultivar harvested from one replicate of control plants and SA treated plants with three concentrations: 0.5, 1 and 5 mM, at harvest and after 21 days of storage at 7 °C. (B) Heatmap of decay incidence for ‘Herminio’ cultivar after 21 days of postharvest storage at 7 °C. Colors in the diagram represent the low or high percentage of incidence, ranging from green to red, respectively; and numbers below each color map represent the different treatments (1 = control; 2 = 0.5 mM SA; 3 = 1 mM SA; 4 = 5 mM SA).

## 4. Discussion

### 4.1. SA Preharvest Treatment Applied at Low Concentration Tested Increases Crop Yield

In the present experiment, control and SA treated plants were grown in a greenhouse under similar climatic and agronomic conditions, as a result the differences between the control and treated plants was just due to the effects of treatments tested. The increase of accumulated crop yield (kg per plant) was higher with 0.5 mM SA treatment than SA at 1 mM concentration, although a negative effect was observed for 5 mM dose. Results also showed that SA treatments at 0.5 mM increased crop yield due to an increase in the number of harvested fruits per plant, which had a higher average fruit weight. In the same way, the effect of 5 mM SA treatment on reducing crop yield was mainly due to a sharp decrease on the number of harvested fruits per plant as well as due to a lower average fruit weight caused by the treatment. Due to the treatments being performed before the beginning of flowering stage, the effect of SA treatment on increasing or reducing fruit number could be mainly due to a direct consequence on flowering rate, set fruit rate or on pepper fruit abscission from the plants, which naturally occurs during the fruit developmental process. Previous work has studied the action of exogenous application of SA at 0.001 mM on flower induction and fructification in habanero pepper plants [33]. Results demonstrated that the number of flowers per plant observed at 80 days after spraying was 1.70-fold higher in SA treated plants than controls, leading into a 40% higher fructification compared with the control.

On the other hand, our results of SA treatment applied at the lowest concentrations on increasing average fruit weight prove that this plant hormone could increase net photo-assimilate production in pepper plants. In fact, Tucuch-Haas et al. [33] also reported that 0.001 mM SA preharvest treatment significantly increased growth and fresh and dry weight of roots, stems, leaves and fruits of *Capsicum chinense* plant species at harvest, mainly due to a positive effect of SA on increasing the uptake of macronutrients and micronutrients that

are allocated in the plant tissues. Therefore, exogenous application of SA may influence a range of plant processes, including stomatal closure, ion uptake and transport [34], membrane permeability [35] and photosynthetic and growth rates [36]. For instance, in the 'California Wonder' sweet pepper, SA has been reported to increase vegetative growth, photosynthetic pigments, mineral content, and endogenous auxin and cytokinin levels, while decreasing abscisic acid levels [37]. Moreover, an increase on Rubisco activity and total yield by SA treatment were also reported in maize and mustard plants [38]. In addition, a possible action of SA on the reduction of pepper fruit abscission during the crop cycle is not discarded as it was previously reported in pomegranate fruit [32].

Nevertheless, our results reported that SA applied at high concentrations (5 mM) can exert the opposite effect, decreasing the yield in pepper plants. Accordingly, salicylates preharvest treatments in table grapes at high concentration, 5 and 10 mM, delayed berry ripening and reduced crop yield, while ripening was accelerated and crop yield increased at lower concentrations [39]. Thus, for the first time, results on crop yield of 'Herminio' pepper plants are reported and depend on applied concentration of SA. Similarly, pepper fruit grown under moderately salt-stressed greenhouse conditions showed positive results in terms of productivity and fruit quality with SA application at a low concentration (0.001 mM) [23]. To our knowledge, the lowest concentration of SA tested in the present experiment (0.5 mM) could be a useful tool to increase crop yield in green pepper plants.

#### 4.2. SA Preharvest Treatment Applied at Low Concentration Tested Improves Fruit Quality Parameters and Functional Quality at Harvest and during Storage

The loss of water in sweet peppers is one of the problems that is generated during storage, leading to fruit firmness changes [40]. Consumers have become more critical in the last decade with fruit quality parameters, taking into account flavor and firmness [41]. In this sense, SA preharvest application at 0.5 mM was the most effective treatment significantly reducing the weight loss of pepper fruits at 21 days of storage at 7 °C. Nevertheless, all SA treatments efficiently increased fruit firmness both at harvest and at the end of postharvest storage. Other authors have observed a significant improvement by 3 mM SA-foliar application on 'Yolo Wonder' pepper cultivar in fruit firmness at harvest [25]. The general positive effects delaying losses of fruit weight and firmness during the postharvest storage experiment of 'Herminio' pepper fruits as a result of applying SA could be attributed to its role on the activities of cell wall modifying enzymes, such as polygalacturonase (PG) and pectin methyl esterase (PME), according to the results reported by Rao et al. [10]. In this study, the authors observed that levels of these enzyme activities were found to be decreased by the SA and CaCl<sub>2</sub> postharvest treatments, suggesting that SA and CaCl<sub>2</sub> delays softening of *Capsicum* fruit and supports brittleness and firmness retention of the fruit flesh. Srivastava and Dwivedi [42] also reported that SA treatment suppresses the cell wall degrading enzyme activities. On the other hand, a key reduction of fruit respiration rate was observed both at harvest and after 21 days of storage by all SA treatments compared to untreated pepper fruits. Perhaps, similar to research reported by Rao et al. [10] in 'Indra' sweet pepper cultivar, the fruits treated with SA could present reduced PG and PME activities that may be due to the antisenescence action and inhibitory effect of SA on ethylene biosynthesis. This effect could delay the activity of enzymes responsible for ripening and resistance to pathogen incidence; therefore, cell wall degradation could be prevented by this preharvest treatment, which in turn facilitated the reduced water loss and lesser fruit respiratory gas exchange. In fact, Rao et al. [10] concluded that SA at 1 and 2 mM applied as a postharvest treatment led to a lower percentage of shrinkage in the treated pepper fruits. Similar results can be observed in 'Herminio' green pepper fruit (Figure 4A), where shrinkage symptoms were observed in control pepper fruits at the end of postharvest storage.

Furthermore, SA foliar application did not affect the color of green pepper fruit at harvest. Nevertheless, losses of green color during storage were delayed by all SA treatments leading to less yellowish pepper fruits and, specially, for those fruits treated with SA at 0.5 mM concentration (Figure 4A). This result of preharvest SA treatment on

avoiding the pepper fruit discoloration during storage could be a consequence of the treatment delaying the fruit senescence process. In this sense, a previous study concluded that SA applied as postharvest treatment extended the shelf-life of sweet pepper fruits by up to 71 days stored at 10 °C [10]. Despite no significant differences being observed on TSS content, all SA treated pepper fruits showed higher levels of TA at harvest. However, the TA content after 21 days of storage was only significantly higher in those pepper fruits harvested from 0.5 and 1 mM SA-treated plants. Other authors have reported that vitamin C, TSS and TA were increased at harvest in 'California Wonder' sweet pepper fruit also as a response to 0.1 mM SA [37]. Hanieh et al. [43] reported that SA application at 0.7 mM also increased TA but decreased TSS in 'Cadia' sweet pepper. Several studies concluded that content of vitamin C, TA and TSS, and total sugars in sweet pepper fruit are cultivar-dependent and influenced by growth conditions [44,45]. Therefore, we can conclude that SA preharvest treatment did not affect the on-plant ripening process of pepper fruit, although it led to pepper fruits with higher levels of organic acids at harvest and during storage.

Phenolics content is a suitable indicator to evaluate environmental stress tolerance and improve plant metabolism, inducing stress tolerance in plants through light or antioxidant protection [46]. For the first time, the effect of SA preharvest treatment of green pepper plants on phenolic content at harvest and during fruit storage has been reported in the present study. Our results showed that SA applied at the three studied concentrations increased the total phenolics content at harvest. However, it would be worth highlighting that 0.5 mM SA was the most effective treatment for improving total phenolic content after 21 days of storage at 7 °C. In Chinese cabbage, Thiruvengadam et al. [47] reported that SA increased the expression of genes codified by enzymes, such as chalcone synthase (CHS) and chalcone isomerase (CHI), which are involved further downstream in the pathway of flavonoids. Other authors have hypothesized on the effects of salicylate treatments on enhancing total phenolic concentration could be due to the activation of phenylalanine ammonia lyase (PAL) activity, which is the main enzyme involved in the biosynthetic phenolic pathway, by these treatments [32,39].

Ultimately, the antioxidant capacity is given by compounds present on hydrophilic and lipophilic fractions. Hydrophilic compounds are mainly ascorbic acid or vitamin C, glutathione (GSH) and phenolic compounds, mainly flavonoids, while the lipophilic ones are mainly chlorophylls, carotenoids and vitamin E [15]. Regarding H-TAA, all preharvest treatments with SA enhanced the TAA of those compounds presented in the hydrophilic fraction at harvest. Nevertheless, the 0.5 mM SA was the most effective one on this functional improvement at the end of postharvest storage, followed by 1 and 5 mM SA treatments. Previous studies have shown that SA at a low concentration (0.001 mM) positively increased vitamin C content in pepper fruits grown in a moderately salt-stressed greenhouse at harvest [23]. Under salt-stress conditions, higher content of ascorbic acid could maintain relatively lower levels of reactive oxygen species (ROS) in pepper fruit, resulting in less damage caused by these ROS since ascorbic acid as an antioxidant plays an important role and protect the plant during oxidative damage by scavenging free radicals and ROS [48]. Other authors have also reported that preharvest treatments with salicylates on pomegranate trees increased total phenolic compound content, as well as ascorbic acid, leading to increases in H-TAA [32]. Therefore, we hypothesize that foliar application of SA to pepper plants could increase the vitamin C content of green pepper fruits, leading to an enhanced H-TAA, as has been observed in terms of total phenolic content.

Chlorophylls are lipophilic-nature pigments, which change during pepper development on the plant and are responsible for the characteristic green color of each pepper cultivar. Nevertheless, chlorophyll degradation by chlorophyllase, which is the enzyme catalyzing the conversion of chlorophyll to its degradation product chlorophyllide, and loss of green color of 'Herminio' pepper fruits are direct consequences of postharvest storage, which are directly as a result of specific relative humidity [49]. Our results showed that SA preharvest treatment at 0.5 mM increased L-TAA of green pepper fruits at harvest and

after postharvest storage. Accordingly, other authors reported that chlorophyll content decreased under drought or salt stress in bell pepper cultivars, but SA spray increased this content [50]. The increase of L-TAA could be mediated by the role of SA on increasing chlorophyll content at harvest and on delaying its deterioration during storage at 7 °C, since the greatest visual aspect in terms of green color was recorded on both sampling dates (at harvest and after 21 days of storage; Figure 4A) in those peppers treated in pre-harvest with SA at a dose of 0.5 mM. Both antioxidant activity fractions could be related to potential health functionality against various chronic non-communicable diseases [51]. Therefore, it is advisable that the green pepper fruits treated with SA at the lowest concentration (0.5 mM) could improve green pepper fruit quality and their content on antioxidant compounds with beneficial health effects, both at harvest and during storage.

#### 4.3. SA Preharvest Treatment Applied at Low Concentration Tested Induces Fruit Tolerance against Decay Incidence during Storage

The lowest percentage (%) of decay was recorded in those green pepper fruits harvested from 0.5 mM SA treatment, followed by SA treatments at 1 and 5 mM concentrations. The induction of green pepper fruit tolerance by SA foliar application could be related to the stimulation of peroxidase and polyphenoloxidase activities, as was reported by Mekawi et al. [21]. These authors found that salicylic acid at 8 mM was the most effective treatment against grey mold caused by *B. cinerea* and for maintaining naturally infected pepper fruits. Accordingly, Li and Zou [52] observed that SA application significantly increased the accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anion (O<sub>2</sub><sup>-</sup>), PAL activity, expression level of PR gene (pathogenesis related protein) in tomato plants, improving fruit resistance against *B. cinerea*.

## 5. Conclusions

The commercial harvest stage of green pepper fruit is prior to full developed physiological stage, which directly influences its postharvest quality. In this sense, this is the first report showing that foliar application of SA in preharvest to green pepper plants has a significant effect on crop yield, fruit quality parameters and functional quality at harvest and after 21 days of storage at 7 °C. The lowest concentration of SA tested (0.5 mM) showed the best results since this treatment increased crop yield, in terms of kg per plant, number of fruits harvested per plant and average fruit weight and fruit quality parameters as well as bioactive compound content at harvest. In addition, this treatment delayed various losses of physico-chemical and functional traits that normally occur during postharvest storage of pepper fruit at non-chilling temperatures, leading to fruit quality maintenance after 21 days of storage. Finally, SA preharvest treatment applied at 0.5 mM was the most effective tool in order to induce pepper fruit tolerance against decay incidence during storage. Thus, SA applied at 0.5 mM could be a safe, useful and natural preharvest strategy to increase crop yield and green pepper fruit quality parameters at harvest and to maintain them during storage. However, more studies are needed in order to elucidate the effect of SA application on the flowering biology of pepper plants that could affect crop yield over the year.

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