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Effect of elicitors and biostimulants on the content of bioactive compounds in raspberry fruits

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Abstract: Raspberry has acquired great interest in human health due to its content of bioactive compounds that provide protection against diseases caused by non-communicable diseases. Bioactive compounds are mainly represented by secondary metabolites such as phenols, anthocyanins, and flavonoids. Biostimulants and elicitors are substances or microorganisms that provide protection and defence to the physiological processes of plants. The present study evaluated the effect of two elicitors (hydrogen peroxide, salicylic acid) and three biostimulants (humic and fulvic acids, glutamic acid, seaweed extracts) on the content of bioactive compounds in raspberry fruits, agronomic and fruit yield parameters in plants. Hydrogen peroxide increased the content of bioactive compounds such as flavonoids, anthocyanins, omega 3 and oleic acid. Salicylic acid increased the content of flavonoids, anthocyanins, and citric acid in raspberry fruits; the number of fruit loaders and fruits per plant was also increased. Humic and fulvic acids, glutamic acid, and glutamic acid combined with seaweed extracts increased the content of flavonoids and anthocyanins, without affecting growth parameters and fruit yield. Glutamic acid and seaweed extracts were the only treatments that increased the content of palmitic acid, while seaweed extracts increased °Brix content in fruits.

Keywords: fulvic acids; humic acids; seaweed extracts; hydrogen peroxide; salicylic acid; oxidative stress

The fruits of the *Rosaceae* family known as berries, have acquired great interest in human health due to their content of bioactive compounds that provide protection against non-communicable diseases (Skrovankova et al. 2015). The red raspberry (*Rubus idaeus* L.) is one of the berries with the greatest therapeutic potential since its bioactive compounds are mainly represented by phenols, anthocyanins and flavonoids (Teng et al. 2017). Additionally, this fruit contains vitamins, long chain

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fatty acids (PUFAs) and organic acids (Skrovankova et al. 2015). Anthocyanins, flavonoids and phenols have properties anti-inflammatory, anti-cancer, anti-vascular and neurodegenerative diseases, among others (Kim, Lee 2020; Speer et al. 2020). Long-chain fatty acids such as omega 3 and 6 are essential fatty acids necessary for brain membranes and their deficiency is associated with cognitive diseases (Dinicolantonio, Keefe 2020; Shrestha et al. 2020). Phenols are the result of the secondary metabolism of plants and eventually, under adverse factors, their synthesis is accelerated since they are responsible for the adaptation of plants, when they are subjected to some type of stress; however, this stressful conditions alter the quality, growth and development of the plants (Teklić et al. 2021).

An alternative to increase the content of bioactive compounds without affecting quality and yield is through induced stimuli by elicitors and/or biostimulants due to their function as signalling mediators (du Jardin 2015; Ahmad et al. 2019; Ali 2021). Biostimulants can be substances or microorganisms that provide protection and defence to the physiological processes of plants (du Jardin 2015); while elicitors induce physiological defence responses in plants (Ali 2021). When plants perceive the signals, they activate pathways at cellular level with the aim of accelerating the production of enzymes and compounds related to antioxidant capacity (Mannino et al. 2021; Teklić et al. 2021), thus, increasing the content of bioactive compounds beneficial to human health.

The present study was conducted to evaluate the effect of two elicitors: hydrogen peroxide and salicylic acid, and three biostimulants of plant origin: Humic and fulvic acids, glutamic acid, and seaweed extracts, on the content of bioactive compounds in raspberry fruits, agronomic and yield parameters in plants.

METHODOLOGY

Plant development. The experiment was carried out in a temperature-controlled greenhouse, located in the Department of Horticulture at Autonomous Agrarian University Antonio Narro in Saltillo, Mexico (25°21'14''N, 101°02'14''W). Raspberry plants (*Rubus* spp.) cultivar XZ28 were established in rigid squared black plastic containers with a volume of 20 L. The growing medium consisted of 100% coconut fibre, which provided the following physical properties, 25% air filled porosity, 69% volumetric moisture content and 94% total porosity. Plant spacing was 4 plants per linear meter with a separation of 1.5 m between lines. The plants were irrigated with a modified Steiner (Steiner 1961) nutrient solution (Table 1), which was applied by a drip irrigation system; the emission capacity of each dripper was 2 L/min, the frequency of irrigation was every 2 to 4 h depending on the prevailing weather conditions and the amount of water applied was until a drainage volume of 25 to 30% was obtained.

On average, air temperature ranged from 15.5 to 23.8 °C and averaged 19.6 °C, and relative humidity ranged from 75% to 88% and averaged 81% throughout the growing season.

Description of treatments. Eight treatments were applied, two elicitors: hydrogen peroxide 50% (10^{-4} M), salicylic acid 99.5% (0.750 g/L), and three biostimulants: humic and fulvic acids (10 mL/L), glutamic acid 99.28 % (0.750 g/L), glutamic acid (0.2 g/L) plus seaweed extract (2.5 mL/L), seaweed extract at 7.5 mL/L – 1 and 15 mL/L – 2, and a control with no biostimulants or elicitors applied. The source of seaweed extracts was Algaenzims® (Palau Bioquim SA de CV, Saltillo, Mexico). The treatments were foliar sprayed, and experimental design was completely random with four replica-

Table 1. Nutrient solution used for soilless culture for growing raspberries under greenhouse conditions

Nutrient	Unit	Concentration
NO_3-N	(meq/L)	5.5
NH_4-N		1
H_2PO_4		1
SO_4		4.5
K		4.5
Ca		5.0
Mg		2.5
Iron	(ppm)	2.0
Boron		0.6
Manganese		0.04
Zinc		0.2
Copper		0.1
Molybdenum		0.05
pH		5.5–6.0
Electrical conductivity	(dS/m)	~1.4

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tions, each replication had three plants, Treatments applications started at flowering stage and continued every 2 weeks, until six applications were completed; considering the first application as day 1, the following applications were done 15, 30, 45, 60 and 75 days after the first one.

Sample processing. Three fruit harvests to process samples for the study were conducted 48 h after the 4^{th} (day 47), 5^{th} (day 62), and 6^{th} (day 77) application of treatments. The fruits were manually harvested at the maturity stage using nitrile gloves protection and the fresh weight was recorded immediately. Then, fruits were transferred to freezer at –80 °C (Thermo Fisher Scientific LLC). Subsequently, the samples were dehydrated with a lyophilizer (FreeZone 2.5 Liter Benchtop Free Dry System, LABCONCO) and then macerated in a mortar.

Phenols content. Total phenols were extracted using a water/ketone (1 : 1) solution based on the methodology by Singleton et al. (1999). Two mL of water/ketone were added to 100 mg of lyophilized tissue and homogenized by vortexing during 10 s and subsequently sonicated for 5 min. The samples were then centrifuged at 12 000 rpm for 10 min at 4 °C and the supernatant obtained was filtered through a 0.45 µm diameter nylon membrane.

Then a 50 µL aliquot were added to 200 µL of Folin Ciocalteu 1M reagent, 500 µL of 20% sodium carbonate and 5 mL of distilled water (reacted for 30 min at 45 °C). The absorbance A_{750} nm was measured by spectrophotometry (Genesis 10s Uv-Vis, Thermo Scientific, Waltham, USA). The phenols were expressed as mg/g of dry weight according to the equation of a straight line, using gallic acid as standard.

Flavonoids. Flavonoid analysis was performed following the method by Zhishen et al. (1999). A 50 mg sample was homogenized with 2 mL of 80% methanol and centrifuged at 4 000 rpm for 10 min at 4 °C. The reaction mixture consisted of 250 µL of the aliquot into a test tube and 75 µL of 5% sodium nitrite ($NaNO_2$). Mixes were vortexed and let stand for 5 min. Then, 150 µL of 10% aluminium chloride and 500 µL of 1 M sodium hydroxide (NaOH) were added subsequently. The absorbance A_{510} nm was measured by spectrophotometry (Genesis 10s Uv-Vis, Thermo Scientific). Using catechin as standard, the flavonoids were expressed as mg/g of dry weight according to the equation of a straight line.

Anthocyanins. Anthocyanin analysis was conducted by the Di Stefano et al. (1989) method. A 100 mg of tissue sample was homogenized with 2 mL of an ethanol/water/concentrated hydrochloric acid (70/29/1) solution. Mixes were vortexed and centrifuged at 12 000 rpm for 10 min. Then, a 250 µL aliquot was taken and placed in a test tube, then 2 mL of ethanol/water/concentrated hydrochloric acid (70/29/1) solution were added subsequently. The absorbance A_{540} nm was measured by spectrophotometry (Genesis 10s Uv-Vis, Thermo Scientific). The anthocyanins were expressed as equivalents of malvidin-3-glucoside mg 100/g of dry weight following the next formula:

$$TA = (A_{540}nm)\ (16.7)\ (dilution)$$

Long chain fatty acid extraction. Fatty acids analysis was performed using the modified technique of Folch et al. (1957). A 50 mg quantity of the material was homogenized with 1 mL of (2 : 1) chloroform-methanol (reacted for 30 min at a temperature of 60 °C). Then 200 µL aliquot was taken and 1 mL of NaOH/MeOH·5 M, 1 mL of dichloromethane and 20 µL of methyl heptadecanoate were added (used as an internal standard), sample reacted for 30 min at 80 °C. Then 1 mL of boron trifluoride was added subsequently (Kang, Wang 2005). Later the organic phase was washed with hexane and filtered through a column with sodium sulfate, the hexene was evaporated with nitrogen and 20 µL of isoctane was added.

Fatty acid quantification. Fatty acids were analysed by GC-MS gas chromatography/mass spectrometry. A 2 µL of isoctane extract was injected on gas chromatograph (Agilent 5890 Series II; Agilent, Foster City, USA) coupled to an MS detector (HP 5972) with Chemstation software (Hewlett-Packard Co.), the SUPELCO SP-2330 column (30 m) was used. The injector temperature was adjusted to 230 °C, using helium as carrier gas. The oven program started at 130 °C. The oven program used in GC-MS was as follows: level 1 held at 190 °C for 5 min, level 2 at 190 °C for 2 min and level 3 at 218 °C for 1 min.

Data were obtained by the Enhaced Data Analysis program, retention times and mass spectra were obtained using automated mass spectral deconvolution and identification system. The identity of the molecules was obtained by comparing the mass spectral of the respective extracts with the mass

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spectra of the library and software National Institute of Standards and Technology, USA.

Fruit quality. Citric acid analysis was performed by (GC/MS) as described above. The total soluble solids (°Brix) were analyzed using a HI 96801 digital refractometer (0–85%).

Growth and fruit yield of raspberry plants. The growth variables of the plants were quantified at the end of the productive cycle. These included the final dimensions of height using a tape measure (Truper Model 12694) and the stem diameter with a calliper (Digital Electronic Vernier Caliper 0–150 Mm De Fibr). The number of leaves, number of fruit loaders and fruit yield per plant were manually quantified. The fruit yield was calculated by the sum of the total number of fruits harvested per plant through the period.

Statistical analysis. Data were analysed by one-way ANOVA using the INFOSTAT statistical package (V2017). To meet the assumptions of the ANOVA data were transformed as needed. The Fisher's LSD test ($P \leq 0.05$) was used for mean separation.

RESULTS

Phenolic compounds. The phenols content on day 47 and 62 (Figure 1A) was not affected by the treatments applied. At day 77, compared to the fruits of control plants, the content of phenolic compounds

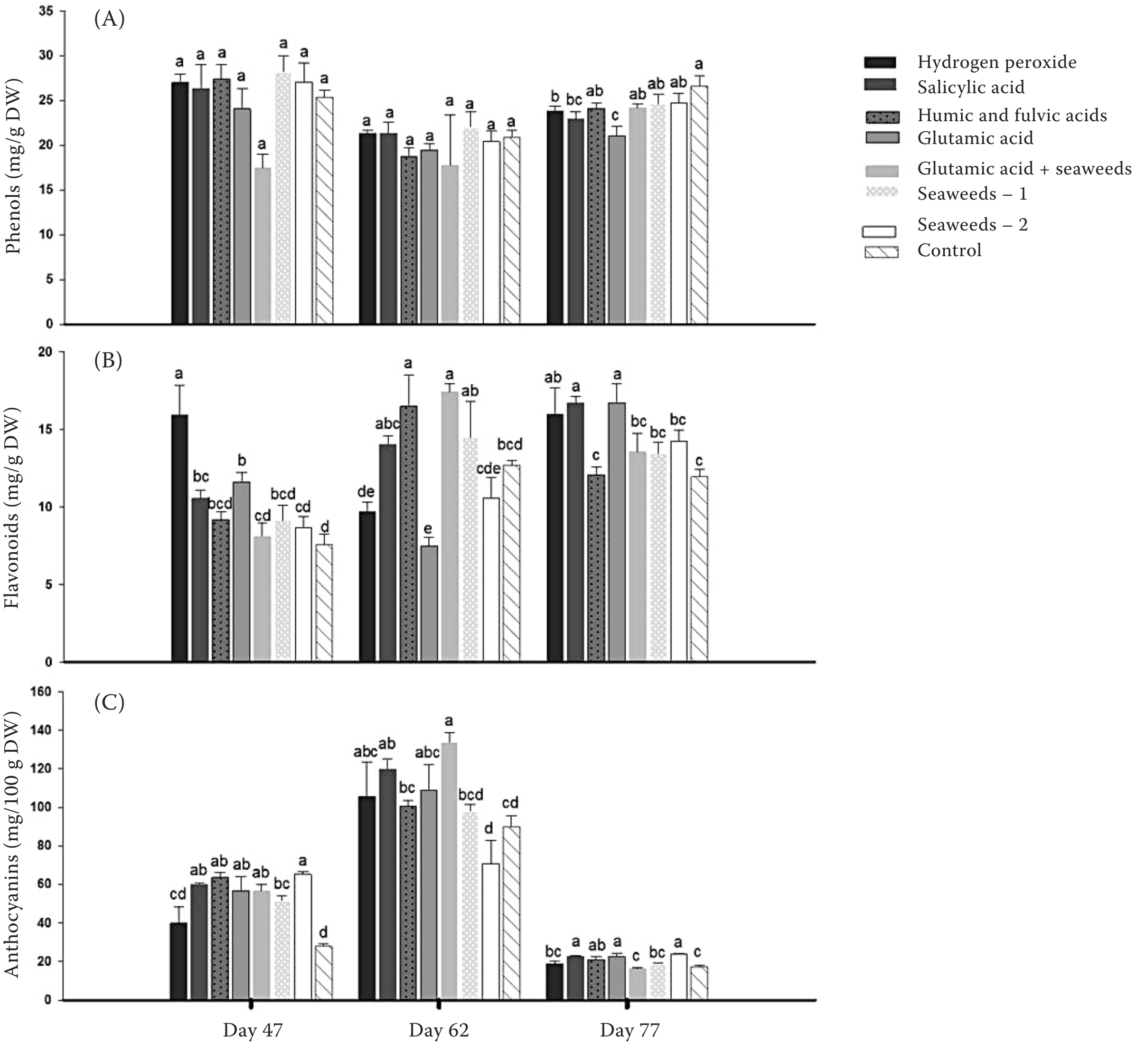

Figure 1. Phenolic compounds from raspberry fruits: phenols (A), flavonoids (B), anthocyanins (C)
The bar on top of every column represents standard error and different letters indicate significant differences, according to Fisher's LSD statistical test ($P \leq 0.05$), the data are the average of 4 repetitions ± standard error

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was decreased by 10.3% and 20.85% when hydrogen peroxide and glutamic acid were sprayed, respectively.

Hydrogen peroxide increased flavonoids in raspberry fruits (Figure 1B) on days 47 and 77 by 110% and 34% respectively. The content of flavonoids increased by 39.12% and 39.79% with salicylic acid applications on days 47 and 77, respectively, while glutamic acid increased the flavonoids content

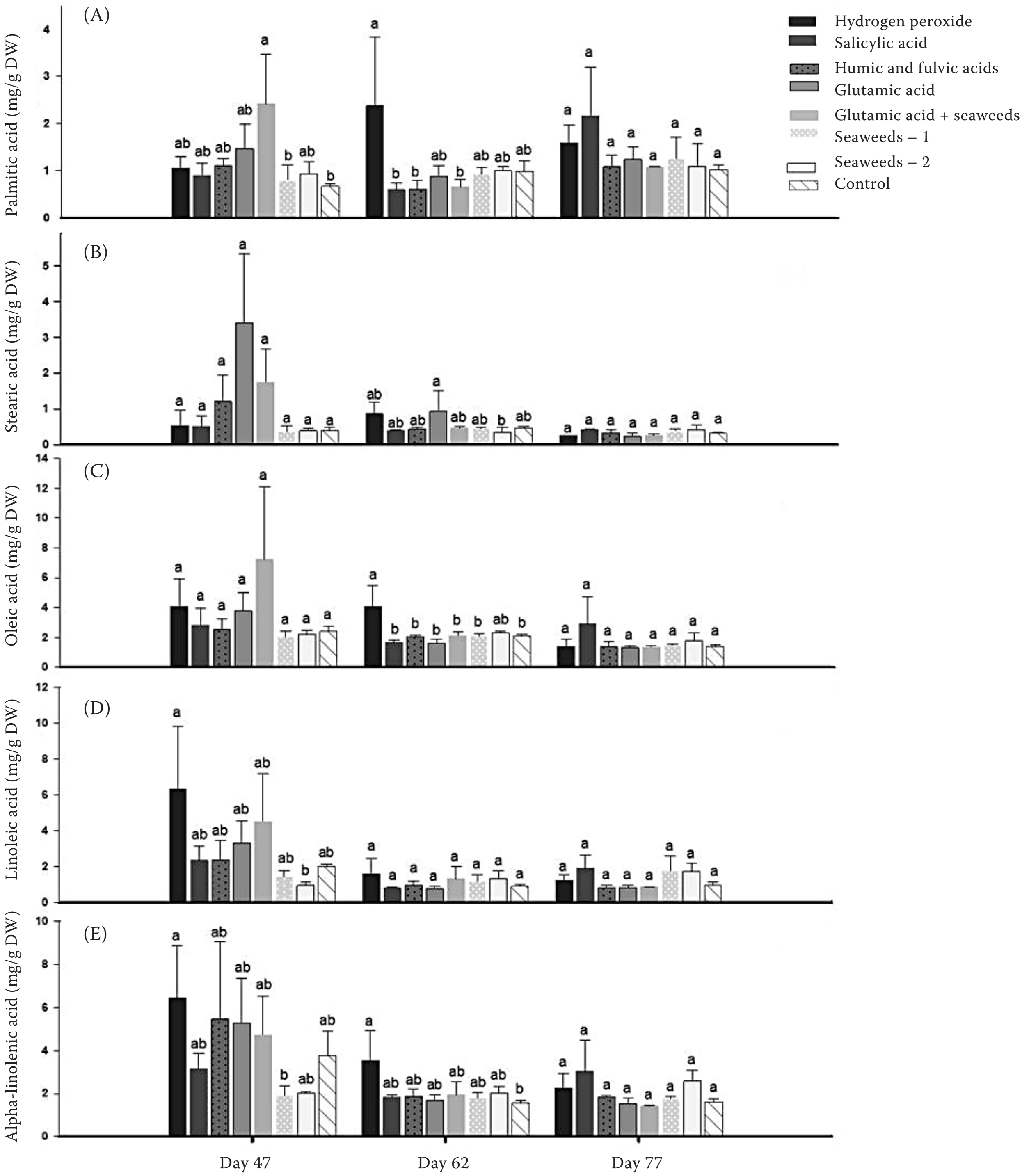

Figure 2. Long chain fatty acids in raspberry fruits: palmitic acid (A), stearic acid (B), oleic acid (C), linoleic acid (D), alpha-linolenic acid (E)

The bar on top of every column represents standard error and different letters indicate significant differences, according to Fisher's LSD statistical test ($P \leq 0.05$), the data are the average of 3 repetitions ± standard error

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in fruits on day 47 and 77 by 52.83% and 39.71%, respectively.

The application of glutamic acid increased the anthocyanins content by 103% and 49% at 47 and 62 days, respectively (Figure 1C) while humic and fulvic acids increased them by 128% and 21% at days 47 and 62, respectively. Anthocyanins content increased by 115%, 34% and 33 % at the 47, 62, and 77 days of sampling with the application of salicylic acid. Glutamic acid with seaweed extracts increased the anthocyanins content by 103% and 49% in the days 47 and 62, respectively. The rest of the treatments did not produce consistent results.

Long chain fatty acids in raspberry fruits. The treatments affected the concentration of long chain fatty acids of raspberry fruits. At day 47 (Figure 2A), the treatment with glutamic acid plus seaweed extracts increased the content of palmitic acid by 259% compared to the control plants without application.

After the 5th and 6th applications, at days 62 and 77, respectively, glutamic acid had no statistically significant effect on palmitic acid, however, it is worth noticing that fruits analysed after the 5th application, at day 62, there was an increase of 140% in palmitic acid when the plants were stimulated by hydrogen peroxide applications.

Regarding the production of stearic acid (Figure 2B), there was no significant effects observed with the application of any treatment in any of the application days. However, it is important to mention that the foliar application of glutamic acid in the day 47, caused an increase of 760% in stearic acid.

Treatment with hydrogen peroxide increased by 92% the content of oleic acid (Figure 2C) on day 62. No significant effects were observed with the application of any treatment on days 47 and 77, however, the application of glutamic acid plus seaweed extracts increased the content of oleic acid by 201% on day 47. The content of linoleic acid was not favored with the application of any treatment (Figure 2D), however, there was an increase of 215% with the application of hydrogen peroxide on day 47.

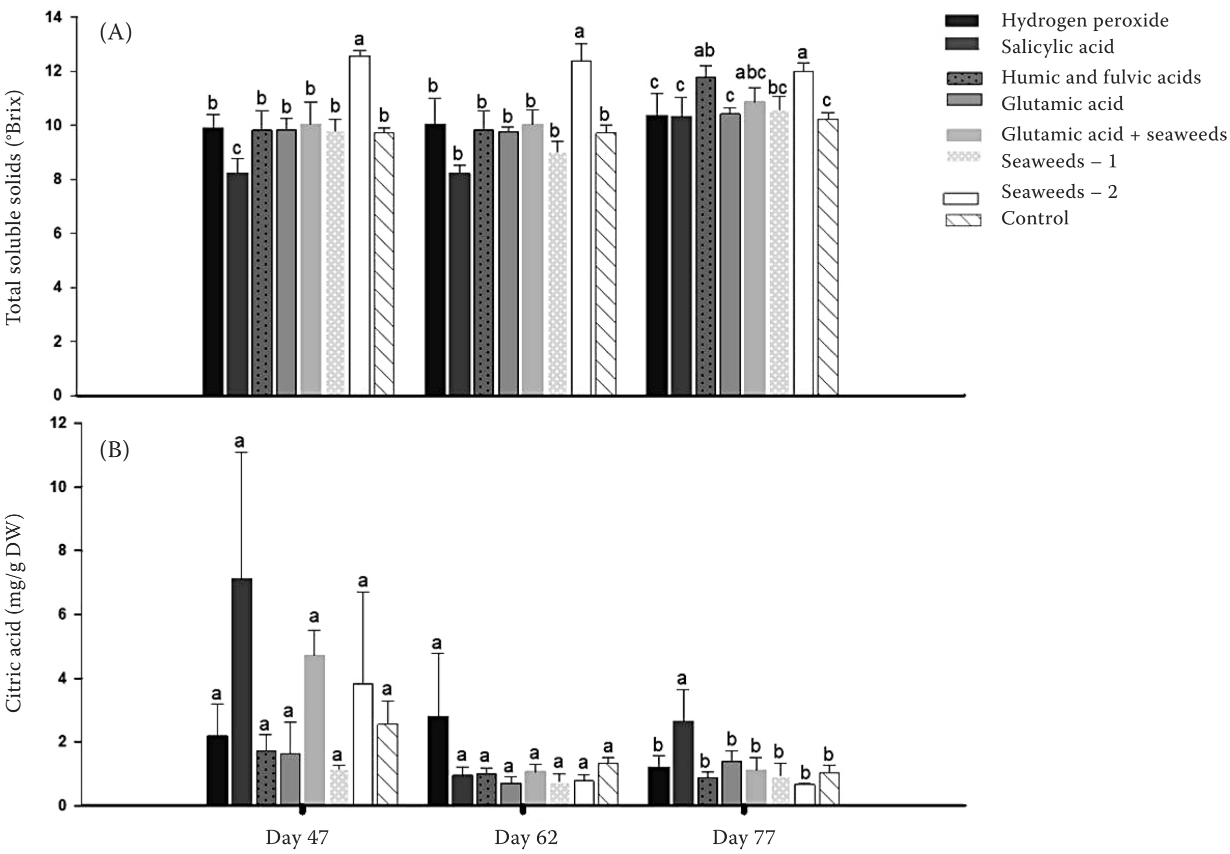

Figure 3. Quality of raspberry fruits: total soluble solids (A), citric acid (B)

The bar on top of every column represents standard error and different letters indicate significant differences, according to Fisher's LSD statistical test ($P \leq 0.05$), the data are the average of 4 repetitions ± standard error

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The content of alpha linoleic acid in fruits of raspberry plants (Figure 2E) on day 62 showed an increase of 127% when plants were treated with hydrogen peroxide. In contrast, there was no significant effects observed with the application of any treatment at the 4th and 6th applications, corresponding to days 47 and 77, respectively; however, at the 4th application (day 47), hydrogen peroxide and humic and fulvic acids caused a gain of 71% and 45%, compared to the control plants, respectively.

Raspberry fruit quality. Treatment with seaweed extracts increased °Brix in raspberry fruits (Figure 3A) at days 47, 62 and 77, by 29%, 27% and 17%, respectively. At day 77, the application of humic and fulvic acids increased °Brix by 15%. The application of biostimulants and elicitors had no effect on citric acid content on days 47 and 62 (Figure 3B). At day 77 the treatment with salicylic acid increased the concentration of citric acid with 154%.

The relationship between °Brix and citric acid is shown in Figure 4. At 47 and 62 days (Figure 4A, B). In general, the application of elicitors (hydrogen peroxide and salicylic acid), increased the acidity of raspberry fruits. Considering the acidity (citric acid) and sweetness (°Brix) of the fruit as 10%; the fruits of the plants treated with hydrogen peroxide 1.8% and 2.18 % of citric acid at 47 and 62 days, respectively. Similarly, fruits stimulated with salicylic acid had 4.6% and 7.2% of citric acid, at the same sampling days. At day 47, the fruits treated with humic and fulvic acids, glutamic acid, glutamic acid plus seaweed extracts, seaweed extracts and control fruits had 1.4%, 3.1%, 3.19%, 2.3% and 2% of citric acid, respectively. At day 77 (Figure 4C) plants treated with salicylic acid were the most acidic, however, the results of these treatments were not consistent. In accordance with the quality standards, the rest of the treatments had an ideal °Brix-citric acid relationship.

Growth and fruit yield of raspberry plants. The treatment with salicylic acid increased the number of fruit carriers by 70% and the number of fruits per plant by 66% compared to the control, in contrast, plants showed a 14% decrease in the number of leaves (Table 2). However, although the stimulation with salicylic acid caused an increase in the number of fruit loaders, and therefore a greater number of fruits, average fruit weight per plant and fruit yield were not significantly affected neither diameter nor height of plants.

The application of biostimulants in raspberry plants promoted flowering and ripening of fruits (Table 2). The plants treated with hydrogen peroxide, glutamic acid and seaweed (T6), induced early flowering compared to the control plants, since the first fruits were collected 37 days after the first application of the treatments (DAA). The first fruits of the plants treated with salicylic acid, marine algae and humic and fulvic acids were collected 39 and 41 DAA. The plants of the treatments with glutamic acid and control were the least precocious (47 DAA).

DISCUSSION

As expected, by inducing controlled oxidative stress in plants with the application of hydrogen peroxide, the content of secondary metabolites

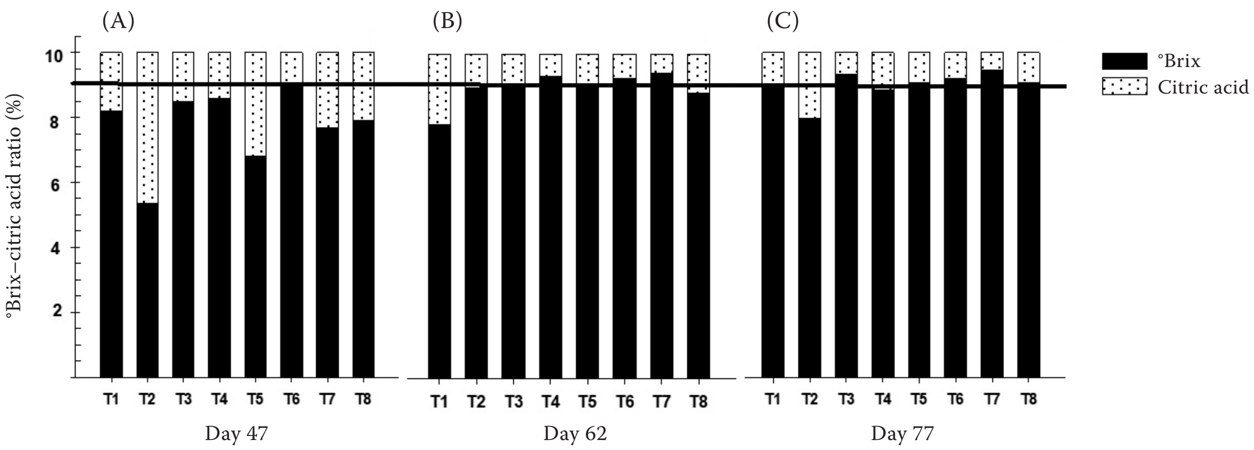

Figure 4. Ratio °Brix–citric acid: day 47 (A), day 62 (B), day 77 (C)

Target range is indicated with the solid line; T1 – 10^{-4} M hydrogen peroxide; T2 – 0.013812 g/L salicylic acid; T3 – 10 mL/L humic and fulvic acids; T4 – 0.750 mg/L glutamic acid; T5 – 0.2 g glutamic acid + 2.5 mL/L Algaenzims; T6 – 7.5 mL/L Algaenzims; T7 – 15 mL/L Algaenzims; T8 – control

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Table 2. Growth and development parameters of raspberry plants

Description of treatments	Days to harvest DDA	Diameter (mm)	No. leaves	Height (cm)	No. fruit loaders	No. fruits	PF	Yield
							(g)	
Hydrogen peroxide 10^{-4} M	37	13.5 ± 1.3^{a}	39 ± 2.3^{ab}	189.9 ± 8.5^{a}	11.7 ± 1.6^{ab}	61.5 ± 7.7^{ab}	4.3 ± 0.2^{a}	268.3 ± 29.6^{a}
Salicylic acid 0.013812 g/L	39	12 ± 0.8^{a}	36.7 ± 0.4^{b}	199.2 ± 11.1^{a}	15.1 ± 2.0^{a}	82 ± 15.3^{a}	4.3 ± 0.2^{a}	367.1 ± 92.6^{a}
Humic and fulvic acids 10 mL/L	41	13.5 ± 1.0^{a}	41.1 ± 1.0^{ab}	193 ± 4.9^{a}	13.4 ± 0.4^{a}	68.6 ± 12.5^{ab}	4.3 ± 0.2^{a}	298.5 ± 67.2^{a}
Glutamic acid 0.750 mg/L	47	13.4 ± 0.6^{a}	42.7 ± 3.2^{a}	200.8 ± 12.6^{a}	14.17 ± 0.8^{a}	61.5 ± 9.4^{ab}	4.6 ± 0.4^{a}	288.9 ± 64.5^{a}
Glutamic acid 0.2 g + Algaenzims 2.5 mL/L	37	12.5 ± 1.4^{a}	38.6 ± 1.2^{ab}	201.6 ± 6.1^{a}	11.7 ± 0.9^{ab}	51.6 ± 14.6^{ab}	4.9 ± 0.4^{a}	240.4 ± 59.5^{a}
Algaenzims 7.5 mL/L	37	13.3 ± 0.7^{a}	41.8 ± 0.8^{ab}	201.2 ± 3.5^{a}	13.1 ± 0.7^{a}	67.7 ± 7.1^{ab}	4.7 ± 0.1^{a}	323.6 ± 34.1^{a}
Algaenzims 15 mL/L	41	14.3 ± 0.9^{a}	41.1 ± 0.7^{ab}	199.2 ± 10.4^{a}	12.6 ± 0.5^{a}	61.8 ± 9.4^{ab}	5.0 ± 0.2^{a}	310.3 ± 51.0^{a}
Control	47	12.9 ± 1.4^{a}	42.7 ± 3.3^{a}	204 ± 9.1^{a}	8.8 ± 1.78^{b}	49.3 ± 6.27^{b}	4.4 ± 0.1^{a}	217.4 ± 26.6^{a}

DDA – days after the first application of stimulants; PF – weight per fruit; different letters in each column indicate significant differences, according to Fisher's LSD statistical test ($P \leq 0.05$), data shown are the mean of 4 replications ± standard error

(flavonoids and anthocyanins) was increased. Since hydrogen peroxide belongs to the reactive oxygen species (ROS), highly reactive molecules that act as signalling mediators and accelerate the secondary metabolism of plants, which is responsible for synthesizing the largest amount of phenolic compounds, carotenoids, vitamins and glutathione (Dumanović et al. 2021). However, high concentrations of ROS cause oxidative stress, damaging subcellular molecules such as DNA, altering cellular homeostasis causing its diffusion and death (Kapoor et al. 2019). Fortunately, a low concentration of ROS activates signalling pathways through phospholipases, releasing calcium (Ca^{+2}) into the cytoplasm. In the cytoplasm, Ca^{+2} is perceived by protein kinases (MAPK), these recognize the information from the second messenger (Ca^{+2}) and activate phosphorylation pathways, followed by gene expression (Lala 2021). The result of the alterations is that the plants accelerate the antioxidant activity which involves enzymes and secondary metabolites, which work together to counteract the oxidation cascade (Dumanović et al. 2021). The mechanism is known as elicitation and is based on inducing stress to speed up the synthesis of bioactive compounds (Lala 2021).

A likely explanation is that the cellular perception of exogenous hydrogen peroxide increased the synthesis of alpha-linolenic acid (omega-3). Since under stress conditions PUFAs are precursors of jasmonic acid and this in turn of phenolic compounds, all this to counteract the damage caused by ROS (Santino et al. 2013). Hydrogen peroxide was the only treatment that increased the content of omega-3 in raspberry fruits, which leads to the hypothesis that elicitation with them improves the dynamic fluidity of plant cells (depends on the degree of unsaturation of lipids). A fluid membrane supports plant cells under conditions of abiotic stress such as temperature and salinity (Los, Murata 2004). Based on the results, the application of hydrogen peroxide could be possibly used to improve the adaptability of plants under stress conditions. Similarly, hydrogen peroxide stimulates flowering in raspberry plants. Early flowering with applications of hydrogen peroxide can be attributed to the fact that this molecule favours photosynthetic capacity and improves growth (Khan et al. 2018). However, more research is still necessary to study the effect of different concentrations of hydrogen peroxide in plants under temperature stress,

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to know if hydrogen peroxide effectively improves cell rigidity. Some studies on the effect of hydrogen peroxide on the content of bioactive compounds in plants are also necessary.

On the other hand, the application of salicylic acid increased the content of flavonoids, anthocyanins, and citric acid in raspberry fruits; in the same way, the number of fruit loaders and the number of fruits per plant increased. Salicylic acid is a phytohormone that acts as a signalling molecule, stimulates many physiological pathways in plants such as ion absorption, membrane permeability, enzymatic activity, disease resistance mechanisms and synthesis of bioactive compounds (Vazirimehr, Rigi 2014; Sariñana-Aldaco et al. 2020). It also improves the quality of fruits by inhibiting the synthesis of ethylene and the development of fungi (Chandra et al. 2007; Mohamed et al. 2018). Salicylic acid acts as an elicitor, its perception by plants favours secondary metabolism, given that it is a plant hormone related to the adaptation of plants against stress (Ali 2021). A possible explanation is that it accelerated the action of the enzyme phenylalanine ammonium lyase (PAL), from which most phenolic compounds are derived (Sengupta et al. 2018). The same can happen with the application of hydrogen peroxide, since both molecules act as elicitors and it has been reported that they manipulate the activity of the PAL enzyme (Mejía-Teniente et al. 2013). Similar results were reported with the application of salicylic acid, since increased the activity of PAL and content of flavonoids and anthocyanins in tomato (Sariñana-Aldaco et al. 2020). On the other hand, several studies have reported early flowering, increase in number of flowers and fruits with foliar application of salicylic acid in strawberry (Mohamed et al. 2018), since it acts as a growth regulator (Martín-Mex et al. 2013). The foregoing agrees with the results of the present work, where the application of salicylic acid increased the number fruit loaders, fruits per plant and promoted early flowering compared to the control plants. This positive effect could be attributed to salicylic acid improving the carbon dioxide (CO_2) assimilation efficiency and the photosynthetic rate (Karlidag et al. 2009). In this study plants treated with salicylic acid produced more fruits compared to control plants, however, they were smaller, and the final fruit yield did not differ statistically.

Based on the results of this work, hydrogen peroxide and salicylic acid could be used as elicitors to produce compounds beneficial for human health in berries, without altering fruit yield. However, it also needs more studies to determine if the effect of different concentrations of hydrogen peroxide and salicylic acid to identify the ideal concentration.

Humic and fulvic acids and glutamic acid, and the combination of glutamic acid with seaweed extracts, favoured the content of bioactive compounds in raspberry fruits. Specifically, treatments significantly increased the content of flavonoids and anthocyanins, without altering growth parameters and crop yield. Glutamic acid plus seaweeds was the only treatment to increase the content of palmitic acid with 259% compared to the control. On the other hand, the application of seaweed extracts was the only treatment that increased °Brix content in raspberry fruits and maintained the quality (relationship between °Brix-citric acid) of the fruit during the three days of sampling. According to raspberry fruits quality standards, their acidity must not exceed 0.7% while the sugars in °Brix must be higher than 9% (Madrid, Beaudry 2020), therefore, the increased of °Brix observed is good for the fruit quality.

Humic and fulvic acids are substances created from the decomposition of plants, microorganisms, and animals, among others (Canellas et al. 2015). Glutamic acid is a protein-forming amino acid (Colla et al. 2015). Seaweed extracts are known for their composition in polysaccharides, minerals, vitamins and growth hormones such as cytokinins and auxins (Battacharyya et al. 2015). Due to the complexity in the chemical composition of biostimulants is difficult to explain which was the mechanism that triggered the increase in the content of bioactive compounds in raspberry fruits (Di Vittori et al. 2018). However, the applied concentrations probably accelerated the phenylpropanoid pathway which implies the accelerating of the PAL enzyme activity. This effect on the PAL and similar results have been reported previously (Afonso et al. 2022), but not in a berry of high economic interest such as raspberry. For example, the treatment with humic substances and glutamic acid is reported to increase the activity of the PAL enzyme (Schiavon et al. 2010; Teixeira et al. 2017). Moreover, the application of algae extracts increased the content of total phenols and flavonoids in broccoli (Lola-Luz et al. 2014). Plant biostimulants influence the hormonal responses of crops, improving the organoleptic characteristics, including colour and °Brix (Rodrigues et al. 2020; Mannino et al. 2021). The colour of raspberry

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fruits is due to anthocyanins and flavonoids (Teng et al. 2017). Biostimulants based on polysaccharides, such as seaweed, can stimulate the production of fatty acids, such as palmitic acid and stearic acid, since polysaccharides are key in the formation of plant structures that provide defence (Farid et al. 2019).

In the present work, the application of biostimulants stimulated early flowering in raspberry plants, without altering fruit yield. In agriculture, biostimulants have been used for the protection of plants under stress conditions and growth promoters since they favour several essential physiological processes, such as ion absorption and photosynthesis (Rouphael, Colla 2020; Teklić et al. 2021).

Based on the evidence, elicitor treatments were the ones that promoted the synthesis of bioactive compounds in raspberry fruits compared to biostimulants treatments and control, by functioning as signalling mediators and activators of secondary metabolism. However, more research is still necessary to study the effect of different concentrations of elicitors and biostimulants on the production of bioactive compounds in berry fruits. The foregoing would be of great importance since berries are currently a fruit with a high economic value (Basri et al. 2021).

CONCLUSION

The application of elicitors and biostimulants can increase the synthesis of bioactive compounds in raspberry fruits without affecting the fruit yield. In addition, probably the application of hydrogen peroxide can be used by increasing the content of essential oils such as oleic acid and omega 3. However, a greater number of investigations are still needed, where the effect of different concentrations of the elicitors and biostimulants used in the present work is studied, to determine the optimal application doses.

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