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1 **Oxidative stress mitigation in horticultural crops using foliar applications of *Ilex paraguariensis***
2 **extract: a dose-dependent study.**

3
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16 **Abstract**

17 Abiotic stress has been shown to induce the formation of reactive oxygen species (ROS) in plant cells.
18 When the level of ROS surpasses the capacity of the endogenous defence mechanism, oxidative stress
19 status is reached, leading to plant damage and a drop in crop productivity. Under oxidative stress
20 conditions, ROS can react with polyunsaturated fatty acids to form oxidized derivatives called
21 phytoprostanes (PhytoPs) and phytofurans (PhytoFs), which are recognized as biomarkers of oxidative
22 damage advance. Modern agriculture proposes the use of biostimulants as a sustainable strategy to
23 alleviate the negative effects of oxidative stress on plants. This work evaluates the dose effect of natural
24 antioxidant extract to mitigate the oxidative-stress deleterious effects in melon and sweet pepper
25 exposed to thermal stress. The plants were sprayed with *Ilex paraguariensis* (IP) aqueous extract in three
26 different concentrations before exposure to abiotic stress. PhytoP and PhytoF levels were determined in
27 the leaves of melon and pepper plants. IP1 and IP2 were effective against oxidative stress in both plants,
28 with IP1 being the most protective one. IP1 decreased the levels of PhytoPs and PhytoFs by roughly 44
29 % in both melon plants and pepper plants. The yield, with IP1, increased by 57 and 39 % in stressed
30 melon and pepper plants, respectively. IP3 foliar application in melon plants induced a pro-oxidant effect
31 rather than the expected mitigating action. However, in sweet pepper plants, IP3 decreased the oxidative
32 stress progress and increased the fruit yield.

33

34 **Keywords** THERMAL STRESS; MELON CROP; SWEET PEPPER CROP; BIOSTIMULANTS;
35 ANTIOXIDANTS; PHYTOPROSTANES; PHYTOFURANS; FRUIT YIELD

36 **Abbreviations**

37 ROS: reactive oxygen species

38 PhytoPs: Phytosteranes

39 PhytoFs: Phytosterols

40 IP: *Ilex paraguariensis*

41 PUFAs: polyunsaturated fatty acids

42 ALA: α -Linolenic acid

43 DAT: Days after transplanting

44 1 INTRODUCTION

45 Nowadays, climate change significantly affects the growth of crops, reducing their productivity.
46 Abiotic stress caused by environmental conditions, including extreme temperatures, high salinity, and
47 hydric restrictions, among others, increases the levels of reactive oxygen species (ROS) in plant cells
48 (Zandalinas, Fritschi & Mittler, 2021). Plants have developed acclimatization mechanisms that give
49 them tolerance to environmental stress conditions. However, when ROS excess cannot be handled by
50 the endogenous defence mechanism, the oxidative stress status is reached. This condition causes cellular
51 damage that leads, in the final stage, to plant death (Raza et al., 2019). Under oxidative stress conditions,
52 ROS can react with polyunsaturated fatty acids (PUFAs), cell membrane constituents, to form oxidized
53 derivatives (Ahmed et al., 2020). In higher plants, most of the oxidized PUFAs are derived from the
54 chemical transformation catalyzed by prooxidant enzymes as lipoxidase and cyclooxygenase. In the last
55 two decades, it has been shown that the non-enzymatic oxidation of PUFAs, such as α -Linolenic acid
56 (ALA), leads to the formation of prostaglandin/jasmonate-like compounds called phytoprostanes
57 (PhytoPs). Besides, furanic structures, named phytofurans (PhytoFs), can also be produced under high
58 oxygen tension. PhytoPs and PhytoFs are two different series of compounds derived from the early
59 stages of the lipid oxidation process. The occurrence of these metabolites in biological systems is
60 increasingly gaining evidence, and they are considered biomarkers of the oxidative stress condition. In
61 a previous study, we determined the PhytoP and PhytoF levels in the leaves of melon plants exposed to
62 thermal stress (Yonny et al., 2016). This was the first study that proposed PhytoFs as oxidative stress
63 biomarkers in stressed plants. Since then, other researchers have published similar results, as Pincirol
64 et al. (2018) who detected PhytoPs and PhytoFs in rice grains from plants exposed to stressful
65 environmental conditions. These metabolites were also found in marine microalgae exposed to hydrogen
66 peroxide (Vigor et al., 2020).

67 Modern agriculture needs to focus on strategies to mitigate the consequences of climate change
68 to ensure food availability for the continuously growing global population. The wide variety of
69 environmental stressors has intensified the search for strategies that contribute to sustainable agriculture
70 from different angles. In this sense, biostimulants are proposed to reach high productivity when the
71 plants are exposed to unfavourable environmental conditions; therefore, these management practices
72 will be in good agreement with global demands for environment-friendly crops (Zulfiqar et al., 2020).
73 Biostimulants are defined as organic and inorganic substances, natural (plant-derived) or synthetic
74 compounds that improve plant productivity as a consequence of the novel or emergent properties of
75 their constituents (Mrid et al., 2021). Biostimulants can function as antioxidants or elicitors for their
76 endogenous biosynthesis. For example, proline, gallic acid and α -tocopherol have been used to mitigate
77 saline stress effects in pepper (Abdelaal et al., 2020), rice (Ozfidan-Konakci, Yildiztugay & Kucukoduk,
78 2015) and onion plants (Semida et al., 2016). It has been reported that exogenous applications on bean
79 plants of liquorice root (Rady et al., 2019) and seaweeds (Deolu-Ajayi et al., 2022) extracts stimulated
80 the growth of seedlings and improved seed vigour, respectively. However, there are still many

81 unanswered questions related to biostimulant treatments, such as the number of applications, application
82 rate (dosage), timing (in the life cycle of the crop), the dose-effect, moment of application, effect
83 durability, among others (Teklić et al., 2021). Therefore, organized, and methodical experiments are
84 necessary to give valuable information to establish adequate crop management practices.

85 The main aim of this work was to evaluate the effect of different doses of *Ilex paraguariensis*
86 (IP) aqueous extract as a biostimulant to mitigate the oxidative stress on melon (*Cucumis melo* L.) and
87 pepper (*Capsicum annuum* L.) crops exposed to high temperatures during their growth. PhytoP and
88 PhytoF profiles were determined in the leaves of these crops to monitor the oxidative stress advance.

89

90 **2 Materials and Methods**

91 *2.1 Reagents and solvents*

92 Water, methanol (MeOH), acetonitrile (ACN), and chloroform were LC-MS grade and provided from
93 Fisher Scientific hexane (Chromasolv, for HPLC, $\geq 99.7\%$), formic acid (FA), ammonia and potassium
94 hydroxide (Fluka, for mass spectrometry 98 %) were provided by Sigma-Aldrich. Solid phase extraction
95 cartridges (SPE) Oasis MAX with mixed polymer phase were purchased (3 mL, 60 mg) from Waters.
96 Milli-Q ultrapure water was used to prepare all the aqueous solutions. The PhytoP analytical standards
97 used were: 9-L₁-PhytoP, *ent*-9-L₁-PhytoP, *ent*-16-*epi*-16-F₁₁-PhytoP, *ent*-9-*epi*-9-F₁₁-PhytoP, *ent*-9-F₁₁-
98 PhytoP, *ent*-16-F₁₁-PhytoP, *ent*-16-B₁-PhytoP, 16-B₁-PhytoP and 16(*RS*)-16-A₁-PhytoP. The PhytoF
99 analytical standards used were: *ent*-16(*RS*)-9-*epi*-ST- Δ^{14} -10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST- Δ^{10} -13-
100 PhytoF and *ent*-16(*RS*)-13-*epi*-ST- Δ^{14} -9-PhytoF. Analytical standards of PhytoPs and all PhytoFs, as
101 well as the internal standard (IS: d₄-10-F₄₁-NeuroP), were synthesized according to procedures
102 previously described (Cuyamendous et al., 2015).

103

104 *2.2 Preparation of biostimulant solution*

105 The extracts were prepared by a decoction of IP-dried leaves, according to Yonny et al. (2016). Different
106 masses of IP leaves were weighted: 10, 20, and 40 g; and 1 L of water was added to constitute IP1, IP2,
107 and IP3 extracts, respectively. The identification and quantification of the major constituents of IP
108 extract were carried out by HPLC-DAD in the same experiment, according to our previous report
109 (Yonny et al. 2018). Chlorogenic (CL) and caffeic (CA) acids were the major phenolic compounds
110 present in the IP extracts. IP1, IP2, and IP3 extracts contain 2.8 ± 0.2 , 4.8 ± 0.4 , and 6.9 ± 0.4 g CL L⁻¹;
111 and 0.15 ± 0.01 , 0.27 ± 0.02 , and 0.39 ± 0.02 g CA L⁻¹, respectively. IP extracts were applied to the
112 melon and pepper plant leaves in the field by spraying 25 plants per litre (40 mL/plant) with a hand
113 sprayer.

114

115 *2.3 Plant samples, crop management and treatments*

116 The field assay was performed in the Experimental Station from I.N.T.A. Santiago del Estero “Francisco
117 Cantos” located at 28°03’S; 64°15’W, Santiago del Estero, Argentina. Seeds of hybrids “Nun de miel”

118 and CLX-479 were used for melon and pepper plants, respectively. These seeds were provided by
119 Rijkzwaan (Buenos Aires, Argentina), and they were sown in a vivarium. When melon or pepper plants
120 had two true leaves, they were transplanted in rows. In the case of the melon crop, these rows were
121 spaced by 1.60 m and 0.70 m between plants, representing a plant density of 8,800 plants ha⁻¹; and in
122 the corresponding assay with pepper plants, the rows were spaced by 1.00 m and 0.40 m between plants,
123 representing a plant density of 25,000 plants ha⁻¹. The soil characterization resulted in 0.06 % N, 64
124 ppm of P, 94 mg L⁻¹ K, and 86 mg L⁻¹ Ca, pH 7.3, and the electric conductivity (EC) was 1.20 dSm⁻¹.
125 The irrigation system used was localized, complementary to rainfall, and one-tape irrigation was placed
126 in each plant row. Droppers were placed every 20 cm on the tape irrigation, and each dropper yielded a
127 rate of 0.8 L h⁻¹. Preventive phytosanitary control was carried out. Both assays (melon and pepper) were
128 constituted of 48 plants each. Each plant group (melon or pepper) was divided into two groups: control
129 and stressed plants. Then, control, as well as the stressed plant of each crop, were divided into four
130 subgroups, three of them with antioxidant applications and one with water. The experimental design
131 was completely randomized, with six plants for each treatment (subgroup). Stressed plants for both crops
132 were covered with plastic of 30 µm thickness 35 days after transplantation (DAT) for five days. The
133 control plants were kept uncovered during the whole experiment. Melon plants covered with plastic
134 (stressed plants) were exposed to maximum temperatures between 38 and 44°C (Figure 1). The
135 maximum environmental temperature range registered was 30–35°C during the mentioned five days
136 (control ones). Concerning pepper plants, both the covered and uncovered groups were protected in turn
137 using a shade structure because these plants are more sensitive to high temperatures. Thus, the covered
138 pepper plants (stressed plants) were exposed to maximum temperatures between 39 and 45°C (see
139 Figure 1), and the maximum environmental temperature range registered for control plants was
140 31–36°C. The foliar spray of the antioxidant solution was performed before plant exposure to stress
141 conditions at 27 and 34 DAT. IP1, IP2, and IP3 extracts were sprayed on both control and stressed
142 plants. The corresponding subgroups without antioxidant applications were sprayed with distilled water
143 instead. Leaves from melon and pepper plants were collected at 40 DAT. The leaves of the different
144 plants were put in 48 separate bags, 6 bags (six plants) for each treatment. Fresh leaves of both melon
145 and pepper plants were lyophilized at 5 µTorr for 72 h, at -80°C. The dried samples were stored at
146 -20°C until analysis. PhytoP and PhytoF extraction of plant tissues was carried out as in Yonny et al.
147 2016. (Yonny et al., 2016)

148

149 *2.4 Total fruit yield*

150 The harvest of melon and pepper fruits started at 90 DAT and 60 DAT, respectively. Fruits were
151 harvested from six plants per treatment, and they were graded into marketable (quality commercial) and
152 non-marketable (with disease symptoms or deformed) categories. The total weights of marketable fruits
153 were registered per plant, and the unmarketable fruits were discarded.

154

155 *2.5 Statistical analyses*

156 Analysis of variance was used to determine significant differences among data using the
157 software INFOSTAT (Di Rienzo et al., 2009). Fisher LSD-test was used to compare means when the
158 effects were found to be significant ($p < 0.05$).

159

160 **3 Results**

161

162 *3.1 PhytoP and PhytoF profiles in melon and sweet pepper leaves*

163 Different PhytoPs and PhytoFs were determined in the leaves of melon and pepper plants
164 exposed to high temperatures. To the best of our knowledge, this is the first time that these metabolites
165 were evaluated in the leaves of sweet pepper plants grown under thermal stress. In both horticultural
166 crops, eight PhytoPs and two PhytoFs were quantified, and their chemical structures are shown in Figure
167 2. B₁- and L₁-PhytoP stereoisomers, could not be quantified individually due to the peak coelution. Thus,
168 PP5 and PP6 were quantified together (B₁-stereoisomers), as well as PP7 and PP8 (L₁-PhytoP
169 stereoisomers). The individual concentrations of PhytoPs and PhytoFs ranged from 1.9-50 $\mu\text{g g}^{-1}$ and
170 24-70 $\mu\text{g g}^{-1}$, and 0.16-3.5 $\mu\text{g g}^{-1}$ and 1.5-4.5 $\mu\text{g g}^{-1}$ in melon and pepper plants, respectively.

171

172 *3.2 Monitoring of ALA oxidized metabolites in thermal-stressed and non-stressed melon plants under*
173 *IP treatment.*

174 Foliar applications of three levels of IP and distilled water were done in both thermal-stressed
175 plants and control ones. Total PhytoP and PhytoF concentrations in control and thermal-stressed samples
176 were statistically different (Figure 3), being 1.5 times higher in stressed plants than in control. Thus,
177 PhytoPs and PhytoFs confirm their role as oxidative stress biomarkers. The PhytoP and PhytoF profiles,
178 which show the variations in the individual concentrations of these oxidative biomarkers in thermal-
179 stressed melon plants for the treatments evaluated, are presented in supplemental material S1.

180 The result of IP application differed in stressed and non-stressed plants (Fig. 3). The IP1 dose
181 was the most effective against oxidative stress since the PhytoP and PhytoF levels in thermal-stressed
182 plants were statistically lower, 42.4 and 47.2 %, respectively, than the corresponding ones in the stressed
183 plants sprayed with water. It should be highlighted that there was no statistical difference in the total
184 concentrations of PhytoPs and PhytoFs between stressed samples sprayed with IP1 and non-stressed
185 samples sprayed with water. Besides, the total PhytoP levels in non-stressed samples sprayed with IP1
186 were statistically lower than in non-stressed samples sprayed with water (31.9 %). The IP1 extract had
187 not only a mitigation effect on oxidative damage but also a biostimulant effect. By contrast, IP2 spraying
188 on thermal-stressed plants decreased PhytoP and PhytoF levels by 26.9 and 30.1 %, respectively,
189 compared to stressed plants sprayed with water, but PhytoP and PhytoF concentrations were similar in
190 non-stressed samples sprayed with IP2 or water. In respect to IP3 dose effect, PhytoP and PhytoF levels
191 in thermally stressed plants were also lower than in stressed plants sprayed with water, 10.9 and 16.8 %, respectively.

192 respectively. However, in non-stressed plants sprayed with IP3, the PhytoP and PhytoF levels were
193 statistically higher than the corresponding ones sprayed with water, 22.6 and 24.1 %, respectively.
194 Moreover, there was no statistical difference in the total PhytoP and PhytoF levels between stressed and
195 non-stressed melon plants sprayed with IP3 and the corresponding PhytoP and PhytoF contents in
196 thermal-stressed plants. Finally, the total PhytoP levels in thermal-stressed samples sprayed with IP3
197 were similar to the corresponding ones in stressed samples sprayed with water.

198

199 *3.3 Monitoring of ALA oxidized metabolites in stressed and non-stressed pepper plants under IP* 200 *treatments.*

201 Different IP doses were sprayed in pepper plants before thermal stress exposure in both control
202 and stressed plants to attenuate their negative effects (Fig. 4). PhytoP and PhytoF concentration levels
203 of stressed and control samples were statistically different, being 53.5 % on average higher the former
204 than the latter. The PhytoP and PhytoF profile that show the variations in the individual concentrations
205 of these oxidative biomarkers in thermal-stressed pepper plants for the treatments evaluated is presented
206 in the supplemental material S2.

207 PhytoP and PhytoF levels in thermal-stressed pepper plants sprayed with IP1 were statistically
208 lower than in stressed plants sprayed with water, being 38.5 and 48.2 %, respectively. PhytoP and
209 PhytoF levels of IP1 treatment were also similar to those of non-stressed plants sprayed with water. This
210 fact demonstrates the oxidative stress mitigation effect (Fig. 4). Moreover, as like in melon plants, the
211 total PhytoP levels in non-stressed samples sprayed with IP1 are statistically lower (20.2 %) than
212 corresponding non-stressed samples sprayed with water. Thus, both effects, oxidative stress mitigation
213 and biostimulant, were also observed in this crop. With respect to IP2 and IP3 treatments, their
214 effectiveness in mitigating oxidative stress decreased but was observed with both doses. Specifically,
215 the PhytoP and PhytoF levels in thermal-stressed pepper plants sprayed with IP2 were statistically lower,
216 31.4 and 35.2 %, respectively, than those in stressed plants without treatment. For the IP3 dose, it should
217 be noted that in this crop, the oxidative stress mitigating effect remained as the PhytoP and PhytoF levels
218 in thermal-stressed pepper plants sprayed were statistically lower, 21.4 and 25.4 %, respectively, than
219 in stressed plants sprayed with water. Concerning non-stressed pepper plants, similar results in PhytoP
220 and PhytoF levels were found among IP2, IP3 and water sprayings.

221

222 *3.4 Evaluation of IP treatment effect in melon and pepper yields from thermal-stressed and non-stressed* 223 *plants*

224 The fruit yields as the total weights of marketable fruits were determined in melon and pepper
225 plants exposed to high temperatures in the presence of different IP doses (or distilled water) compared
226 to the corresponding controls (non-stressed plants). It should be noted that this is the first report of
227 productivity parameters in thermal-stressed melon and pepper evaluated under IP biostimulant effect
228 (Fig. 5 and 6).

229 In terms of melon (Fig. 5), the mean yield in thermally stressed plants sprayed with water was
230 statistically lower (37 %) than the corresponding value in non-stressed plants. There was no statistical
231 difference between the yields of stressed plants sprayed with IP1 and non-stressed plants (sprayed with
232 water). The former was only 1.2 % lower than the latter but 57 % higher than the thermal-stressed plants
233 without IP. Thus, the mitigation of the negative effect of the thermal (and oxidative) stress of this
234 productivity parameter was also observed. Furthermore, the yields of non-stressed plants sprayed with
235 IP1 were slightly higher (10 %) than those of non-stressed plants without IP extract. This finding gives
236 another probe of the biostimulant effect of IP extract at the first dose evaluated. Considering IP2 and
237 IP3 foliar applications, the results showed that there were statistical differences between the yields of
238 stressed plants sprayed with these doses and the corresponding ones in and non-stressed plants (sprayed
239 with water), being 18 and 43 % lower the former than the latter, respectively. The spraying of non-
240 stressed samples with IP2 decreased the fruit yield by 5 % in relation to the non-stressed plants sprayed
241 with water, while with IP3, this decrease was 38 %. Finally, IP2 spraying of thermally stressed samples
242 increased fruit yield with 29 % compared to water-sprayed stressed plants. In contrast, IP3 decreased by
243 10 % compared to water-sprayed stressed plants. Therefore, IP3 spraying was slightly harmful to the
244 melon crop, rather than beneficial.

245 Considering the fruit yields of the pepper crop (Figure 6), the mean yield in thermally stressed
246 plants was statistically lower (21 %) than the corresponding value in non-stressed plants. There were
247 statistical differences between the yield of thermal-stressed plants sprayed with IP1 and non-stressed
248 plants (sprayed with water), being the former 9.1 % higher than the latter, and 39 % higher than the yield
249 in thermal-stressed plants without IP. Therefore, as like in melon plants, IP1 dose also had both stress
250 mitigation and biostimulant effects in the pepper plants exposed to thermal stress during their growth.
251 Regarding IP2, there were no statistical differences between the yield of stressed plants sprayed with
252 this dose and non-stressed plants (sprayed with water), even the former was only 6.6 % lower than the
253 latter and 16 % higher than the yield in thermal-stressed plants without IP. Finally, the results concerning
254 to IP3 foliar application showed a similar behavior that IP2, because the yield of stressed plants sprayed
255 with this dose is comparable to yield of non-stressed plants without IP, being the former 8.3 % lower
256 than the latter and 14 % higher than the yield in thermal-stressed plants without IP. Thus, in the pepper
257 crop, IP2 and IP3 doses also had a mitigating effect.

258

259 **4 Discussion**

260 *4.1 PhytoP and Phyto F levels in melon and pepper plants*

261 The PhytoP and PhytoF levels determined in melon leaves in the present work were between 15
262 and 185 times higher than those previously reported (Yonny et al., 2016). This fact can be explained
263 considering that the melon plant hybrid used in the present work was *Nun de miel*; while, *Sweet ball* and
264 *Early spring* hybrids were those used in the experiment held in 2016, showing the dependence on the

265 plant genotypes. Other studies reported the same situation in other plant species. Pinciroli et al. (2018)
266 reported, in rice grain, 0.19 $\mu\text{g g}^{-1}$ of PhytoPs in ‘H475-3-1-1-2’ genotype and 0.004 $\mu\text{g g}^{-1}$ of PhytoPs
267 in ‘H484-9-1’ genotype; and 0.025 $\mu\text{g g}^{-1}$ of PhytoFs in ‘H458-41-1-1-1’ genotype and 0.002 $\mu\text{g g}^{-1}$ of
268 PhytoFs in ‘H484-9-1’ genotype. Vigor et al. (2020), working with microalgae, reported extreme PhytoP
269 values of 2.2 $\mu\text{g g}^{-1}$ in *Rhodomonas salina* and 0.0008 $\mu\text{g g}^{-1}$ in *Phaeodactylum tricornutum*, and extreme
270 PhytoF values of 2.2 $\mu\text{g g}^{-1}$ in *Rhodomonas salina* and 0.001 $\mu\text{g g}^{-1}$ in *Phaeodactylum tricornutum*.

271 Regarding the PhytoP and PhytoF profiles measured in stress melon and pepper plant
272 (Supplementary Material S1 and S2, respectively), most of the ALA oxidation products found are
273 consistent with those described in our previous report melon leaves (Yonny et al., 2016), as well as in
274 other plant systems, such as herb leaves (Imbusch & Muller, 2000), rice grain (Pinciroli et al., 2018)
275 and microalgae (Vigor et al., 2020). However, compared with our previous study on melon leaves
276 (Yonny et al., 2016), in this work, a new PhytoF molecule was found. Specifically in the present work,
277 the *ent-16(RS)-13-epi-ST- Δ^{14} -9-PhytoF* (PF1) was detected and quantified for the first time in a plant
278 system. In contrast, the *ent-9(RS)-12-epi-ST- Δ^{10} -13-PhytoF*, detected and quantified as mentioned, in
279 our previous study, in the present report, it was detected but its level was not enough to reach the
280 methodology limit of quantification and so, it could not be quantified. This situation is another
281 consequence of working with different genotypes or varieties from the same crop as it was explained
282 and discussed before.

283 For the quantification of PhytoPs and PhytoFs in pepper leaves, there are no previous studies,
284 and this is the first report. The PhytoP and PhytoF profiles and concentration ranges are similar to those
285 found in melon leaves, this an important finding of this work since a common pattern of OS biomarkers
286 could be found in two different species and different hybrids of the same species when exposed to similar
287 abiotic stress conditions.

288 Regarding to thermal stress effect in melon and pepper plant yields, the decrease percentages
289 registered in stressful conditions, were 37 and 21 % respectively. These results can be explained by
290 considering that at temperatures above 40 °C, these horticultural crops face heat damage and severe
291 water loss due to excessive transpiration and evaporation, therefore, it limits physiological processes
292 such as photosynthesis and respiration, which substantially decrease the crop yields (Mittler, Finka, &
293 Goloubinoff, 2012). In this sense, it was reported that the horticultural crop yields decrease 40-50 %
294 because of the thermal stress induced principally by the climate change (Saqib et al., 2022).

295

296 4.2 The biostimulant dose effect on melon plants exposed to thermal stress.

297 Melon plants were exposed to high temperatures as stressing factor. An antioxidant extract was
298 sprayed on these plants in different doses to determine their effect to reduce or delay the oxidative stress
299 condition. The IP1 dose was the most effective against thermal (and oxidative) stress in the melon plant
300 because this dose, under thermal stress conditions, decreased PhytoP and PhytoF levels and increased
301 the total fruit weight per plant in percentages equal to 44.8 and 57 %, respectively. The decrease in the

302 levels of biomarkers of oxidative stress was similar to, or even slightly higher than the value reported
303 (37 %) in our previous work (Yonny et al., 2016). Regarding the increase in yield, the obtained value
304 was higher than that reported by Amarasinghe et al. (2022) in melon plants exposed to high temperatures
305 after the foliar application of brassinoesteroid extract (12.5 %).

306 By contrast to IP1, IP3 extract application was found to be prooxidant because when this extract
307 dose was applied to control samples (without stressful factor), the PhytoP and PhytoF levels increase
308 instead of decreasing compared to the plant sprayed with water (**Figure 3**). In addition to that, there
309 were no statistical differences between the levels of PhytoPs and PhytoFs corresponding to the
310 unstressed and stressed melon plants, both sprayed with IP3. This potential pro-oxidant effect could also
311 be attributed to a too high concentration of phenolic compound present in the sprayed extracts (Yonny
312 et al., 2018). These phenolic compounds have well-recognised antioxidant activity, but it has also been
313 reported that they can have a pro-oxidant effect at high concentrations (Sakihama et al., 2002). The
314 mentioned pro-oxidant effect was also observed in fruit yield because this productivity parameter in
315 thermal stressed plants sprayed with IP3 decreased 43 % in respect to non-stressed plants (without IP),
316 being higher than the obtained in thermal stressed plants sprayed with water in respect to non-stressed
317 plants also sprayed with water (37 %). These results evidence the importance of a dose effect experiment,
318 to establish the safe concentration for biostimulant applications that avoid the change of behaviour to an
319 oxidative stress promoter.

320

321 *4.3 The biostimulant dose effect on pepper plants exposed to thermal stress.*

322 Pepper plants were exposed to high temperatures after biostimulant applications. Both, stressed
323 and non-stressed plants were sprayed with the biostimulant doses in the assay. PhytoP and PhytoF levels
324 were significantly higher in the leaves of stressed plants than in those of non-stressed ones. PhytoP and
325 PhytoF levels were evaluated for the first time to monitor the oxidative stress status of the pepper plant
326 after exposure to thermal stress. Therefore, considering the results obtained, PhytoPs and PhytoFs were
327 also found to be biomarkers of the progression of oxidative stress in this crop. IP1 was determined as
328 the optimum dose for this crop as well as that for melon plants; besides, PhytoP and PhytoF levels
329 decreased in similar percentages to those observed in melon plants. Although, their profile presented
330 variations. This situation suggests that each species responds in a specific way to the same stressing
331 condition within a common pattern as horticultural crops. Unlike what happens in the melon crop, in
332 pepper plants sprayed with IP2 and IP3, the evaluated concentrations of PhytoPs and PhytoFs were
333 statistically different from stressed plants sprayed with water. Thus, it could be concluded that to pepper
334 plants, the IP extract in the maximum dose assayed was still effective to delay the oxidative stress
335 process generated by thermal stress. Although, the percentage decreases of PhytoP and PhytoF were
336 lower than the corresponding ones found in melon plants. Thus, the biostimulant foliar application could
337 be a useful crop management practice to fortify the defense mechanisms of the pepper plant, when it is
338 cultivated in its normal season when high temperatures are expected.

339 The oxidative stress mitigation effects of IP1-IP3 doses were consistent with the corresponding
340 effects on fruit yield as a productivity parameter. In this sense, the total fruit weights per plant in thermal
341 stressed plants sprayed with IP1, IP2 and IP3 were 39, 16 and 14 % higher than the corresponding ones
342 in thermal-stressed plants sprayed with water, respectively. Thus, the biostimulant foliar application
343 could be a useful crop management practice to fortify the defense mechanisms of the sweet pepper plant,
344 when it is cultivated in its normal season when high temperatures are expected. The increase in yield
345 reached with the IP1-IP3 extracts was similar or even higher than that reported by Paradikovic et al.
346 (2013), who registered an increase of 14 % in pepper plant yield because of foliar application with four
347 commercial biostimulants based on a mixture of amino acids, polysaccharides, and organic acids as
348 active compounds. The mentioned study was performed in a hot summer season, when high
349 temperatures in a greenhouse caused physiological stress in plants.

350 Finally, the mentioned results showed that the IP active compounds were capable to mitigate
351 the oxidative stress, reducing cell damage caused by thermal stress; moreover, biostimulants effects
352 were observed in both, melon and pepper crops. It has been described that biostimulants activate plant
353 endogenous defence mechanisms producing a faster recovery of plants when they were exposed to
354 abiotic and biotic stresses (Cardonia et al., 2018; Yahkin et al., 2017). However, these activation
355 mechanisms are often difficult to identify, and they are still under research (du Jardin, Xu & Geelen, ,
356 2020).

357 The dose of biostimulant that is most effective to activate the plant's defense mechanisms may
358 vary depending on the cultivated species. Further studies on the metabolic pathways activated in the
359 presence of biostimulants for each species during their biochemical response to stress are important to
360 understand the underlying mechanisms of action.

361

362 **Conclusion**

363 In the present work, an eco-friendly crop management practice was assayed to mitigate the
364 oxidative stress negative effects on melon and sweet pepper plants of the exposure to high temperatures.
365 The biochemical response of stressed plants previously treated with three different doses of IP extracts
366 was evaluated in terms of early-stage biomarkers of oxidative stress. Moreover, the total fruit yields
367 were also measured. The most convenient IP doses to mitigate the oxidative stress progress and increase
368 the crop production under thermal stress conditions, were found for both, melon and pepper plants.
369 Besides, a prooxidant effect of IP extract found in melon plants exposed to thermal stress is reported
370 here for the first time. These effects, with the IP3 dose, not only increased PhytoP and PhytoF levels,
371 but also decreased the yield of the melon plant. Thus, it could be highlighted that crop yields had a
372 similar trend that the oxidative stress markers levels, which were determined before the harvesting time.
373 This situation suggests that the evaluation of oxidative stress in advance, during plant growth, could
374 give an excellent opportunity to decide if introduce or not introduce some agronomic practise, such as
375 biostimulant application, to fortify the defences of the endogenous plant mechanisms defences before

376 the harvested. In this way, the sustainable management practice propose here will have more possibilities
377 to be effective in order to set a defence plant stress response and anticipate the environmental changes
378 that might affect plant growth irreversibly. These findings become important to mitigate losses in crop
379 productivity in the context of climate change in which crops will have to grow in increasingly
380 unfavourable conditions. Finally, to take advantage of this promising agricultural management practice
381 in warmer years due to climate change, specific conditions should be evaluated for different crops and
382 their hybrids under different abiotic stresses.

383

384 **Author contributions**

385 **Melisa E. Yonny:** Conceptualization, Methodology, Investigation, Writing-Original Draft, Formal
386 analysis, Funding acquisition. **Maria L. Toscano Adamo:** Investigation. **Ariel Rodríguez Torresi:**
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398

399 **Data availability statement**

400 The data that support the findings of this study are available from the corresponding author upon
401 reasonable request.

402

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478 **Figure Captions**

479 **Figure 1.** Evolution of the maximum temperatures during the growth cycles of melon and pepper plants
480 with or without plastic coerture during the thermal stress period, control and thermal stressed plants,
481 respectively.

482
483 **Figure 2.** Chemical structures and nomenclature of PhytoPs and PhytoFs as early oxidative stress
484 advance biomarkers.

485
486 **Figure 3.** PhytoP and PhytoF total contents in control and thermal stressed melon plants in response to
487 different IP dose. Bars represent means \pm SD (n = 6). Different letters show statistically different mean
488 oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests. Lowercase
489 letters represent statistically different PhytoP contents. Capital letters represent statistically different
490 PhytoP contents.

491
492 **Figure 4.** PhytoP and PhytoF total contents in control and thermal stressed pepper plants in response to
493 different IP dose. Bars represent means \pm SD (n = 6). Different letters show statistically different mean
494 oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests. Lowercase
495 letters represent statistically different PhytoP contents. Capital letters represent statistically different
496 mean PhytoP contents.

497
498 **Figure 5.** Effect on fruit yield of the exogenous IP application in control and thermal stressed melon
499 plants. Bars represent means \pm standard deviation (n = 6 in each crop). Different letters show statistically
500 different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer
501 tests.

502
503 **Figure 6.** Effect on fruit yield of the exogenous IP application in control and thermal stressed pepper
504 plants. Bars represent means \pm standard deviation (n = 6 in each crop). Different letters show statistically
505 different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer
506 tests.

507
508 **Supplementary material**
509 **S1-** PhytoP and PhytoF profiles in control and thermal stressed melon plants in response to different IP
510 dose. Bars represent means \pm standard deviation (n = 6). Different letters show statistically different
511 mean oxidative stress biomarkers levels ($P < 0.05$) according to ANOVA and LSD Fischer tests.

512 **S2-** PhytoP and PhytoF profiles in control and thermal stressed pepper plants in response to different IP
513 dose. Bars represent means \pm standard deviation (n = 6). Different letters show statistically different
514 mean oxidative stress biomarkers levels (P<0.05) according to ANOVA and LSD Fischer tests.
515

516 **Highlights**

- 517 • Eco-friendly strategies to mitigate heat stress were tried in horticultural crops.
- 518 • Phytoprostanes and phytofurans were established as biomarkers in stressed plants.
- 519 • IP effect on fruit yields of stressed melon and pepper plants was dose dependent.
- 520 • IP1 extract was the most effective dose to reduce stress biomarkers for both crops.
- 521 • IP1 was the most effective dose to increase the fruit yield for both crops.
- 522 • Prooxidant effect on melon crop was found at IP3 extract dose.

523 **Statement**

524 Extreme high temperatures induce abiotic stress in melon and pepper plants, increase oxidative
525 stress biomarker levels and decrease plant yields. The biostimulant foliar application with *Ilex*
526 *paraguariensis* extracts to these plants, before plant exposure to stress, attenuate their negative
527 effects according to the dose sprayed.















