Research Paper

Plant protein hydrolysate and arbuscular mycorrhizal fungi synergistically orchestrate eggplant tolerance to iodine supply: A two-year study

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ABSTRACT

Biofortification is a promising strategy to overcome iodine (I) deficiencies in the world population. However, since iodine is not essential for plants, its administration may cause phytotoxicity issues. The objective of this work was to evaluate the effects of two biostimulants [arbuscular mycorrhizal fungi (AM) and plant protein hydrolysates (PH)], used alone or in combination, on eggplant plants treated with three different I doses (0, 300 or 600 mg L^{-1}). Results underlined that the highest I dosage significantly reduced plant growth, yield, fruit dry matter content, chlorophylls and stomatal conductance compared to the control. Whereas, I increased total anthocyanins, chlorogenic acid, antioxidant activity, fruit I concentration, proline, H$_2$O$_2$ and malondialdehyde of the biofortified plants compared to non-biofortified plants. The biostimulants enhanced eggplant growth and yield, fruit dry matter content, chlorophylls, total anthocyanins and chlorogenic acid compared to the control, especially when both biostimulants were supplied. Interestingly, it was often recorded an interaction between I and biostimulants, suggesting that PH and AM had a buffer effect on I toxicity, specifically when combined (PH+AM). Overall, our study pointed out that the mutual use of microbial (AM) and non-microbial (PH) bio-stimulants and the application of 300 mg I L^{-1} might be an helpful approach to relieve the detrimental effects of high I dosages and, simultaneously, to increase crop yield and fruit quality of eggplant.

1. Introduction

Iodine (I) is considered an essential micronutrient for humans, since it is implicated in thyroid regulation and thyroid hormones production (Farebrother et al., 2019). Iodine deficiency can cause serious health problems, such as goitre and impaired cognitive development in children (Hetzel, 2016). Consequently, the fortification of plants with I (biofortification) can improve its intake in the human diet. However, the volatility of I in biofortified vegetables is critical as it can influence the effectiveness of biofortification and, consequently, the nutritional intake for humans (Fuge and Johnson, 2015). Indeed, during cooking or food

storage, I can undergo volatilisation processes, i.e. it can be released in gaseous form and, thus be lost from the food (Zhang et al., 2023). However, there are reports (Caffagni et al., 2012; Comandini et al., 2013) highlighting that boiling, baking and heating are appropriate procedures to preserve I in biofortified crops, such as potatoes, tomatoes or carrots. Though, to enhance the amount of I in vegetables, it is essential to provide high amounts of I via fertigation or foliar spray (Sabatino et al., 2021). However, high dosages of I could be deleterious for plants, causing toxicity and damage of cell structures and impairing growth (Welch and Shuman, 2011; Consentino et al., 2023). Plants can absorb I from the soil, stems or leaves, however, its mobility is related to

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environmental circumstances, such as soil pH, composition, texture, and redox potential (Medrano-Macías et al., 2016). Zhang et al. (2023) stated that high concentration of I alters the normal growth and development of plants, causing physiological disorders. Additionally, Incrocci et al. (2019) found a reduction of plant height, leaf area and dry matter in sweet basil plants when KI is applied at dosages higher than 50 mM. Moreover, leaf alteration (chlorosis and burns) was observed in tomato plants supplied with I (Landini et al., 2011). As underlined by Kiferle et al. (2019), the toxic effects caused by I can be related to the ROS oxidative stress. When ROS concentrations go above the optimal operational range of the antioxidant system, lipid peroxidation, protein oxidation and enzyme deactivation occur (Sahu et al., 2022). Even though the exact process is not fully knew, it seems that high I concentration negatively affects plant performances via the increase of malondialdehyde in plant tissues (Zhang et al., 2023). Furthermore, it was reported that an excess of I can also influence the biosynthesis of chlorophyll (Kiferle et al., 2021) and, consequently, the photosynthetic rate (Blasco et al., 2011). Even though the process is not entirely clear, it is assumed that the formation of iodinated proteins or the contribution of I act as an inducing factor in protein production (Zhang et al., 2023).

Throughout the last decade, there has been an up-surging concern in the use of natural products with low environmental impact and, at the same time, capable of enhancing plant performance. Plant biostimulants are natural products that stimulate plant metabolism by increasing the resources use efficiency and the resistance to biotic and abiotic stresses, such as those caused by nutrients (Rouphael and Colla, 2020; Sabatino et al., 2021; Consentino et al., 2022). Biostimulants can be classified into microbial and non-microbial; among the microbials, arbuscular mycorrhizal fungi (AM) are the most broadly employed in agriculture for their helpful influences on plants (Rouphael et al., 2015). These fungi form a symbiosis with plant roots through a dense system of hyphae, which are used to communicate and exchange nutrients with plants (Giovannetti et al., 2001). This strict interconnection between plants and AM has many positive impacts on plants, for instance improved mineral uptake, defence against pathogens and increased crop yield and quality (Begum et al., 2019). Rouphael et al. (2015) stated that AM markedly affect plant response to plant abiotic stress (drought, salinity, high temperature, mineral toxicity, etc.) modulating their tolerance. Furthermore, there are reports underlining that AM increase plant tolerance to mineral elements excess via different strategies like selective nutrient uptake, chelation of toxic elements and production of enzymes and antioxidant molecules which can protect plants from the elements' toxicity symptoms (Diagne et al., 2020; Rouphael et al., 2015).

Furthermore, non-microbial biostimulants - such as protein hydrolysates (PH) - have been often employed in agriculture. This class of biostimulants are derived by protein hydrolysis, a process that breaks long protein chains into smaller peptides and amino acids (Colla et al., 2015). The use of these products (via soil or leaves) has several benefits to plants. Moreover, according to Colla et al. (2015), some of the peptides enclosed in PH act as signalling molecule, having hormone-like activity. Interestingly, it was reported that protein hydrolysates can be beneficial to withstand environmental stresses, such as those prompted by extreme temperatures, drought, or minerals (Tuteja, 2007). However, it was showed that the impacts of these biostimulants mainly differ on their production procedure (Colla et al., 2015). For animal-derived protein hydrolysates, a high temperature chemical hydrolysis process is used, which in turn leads to the degradation of certain amino acids such as tryptophan. For plant-derived protein hydrolysates, a low temperature enzymatic hydrolysis process has a positive effect on the maintenance of specific important amino acids. As a result, plant-derived protein hydrolysates are more effective and safer than those of animal origin (Consentino et al., 2020).

Eggplant (*Solanum melongena* L.) is a largely used vegetable foodstuff in the Mediterranean diet. Among the European Countries, Italy is the main eggplant producer through complementary growing cycles in open field (spring-summer period) and under protected structures (fall-winter period) (Consentino et al., 2022a). Eggplant fruits have a high content of chlorogenic acid, anthocyanin pigments and/or nasunin (Mennella et al., 2012). However, the fruit proximal composition predominantly differs depending on genotype, growing environment and agronomic practices (Radicetti et al., 2016).

Since in literature there are no information on the mutual effects of I supply and biostimulants application on eggplant, and taking into account that microbial and non-microbial biostimulants, supplied alone or combined, are constantly useful to increase both, I plant tolerance and I concentration, the purpose of the present investigation was to assess the effect of three I doses (0, 300 or 600 mg/L) and four biostimulant treatments (control, PH, AM or PH+AM) on 'Birgah' F_1 in an open field eggplant crop grown for two consecutive years. The findings of this study might be beneficial to reduce mineral malnourishment in humans, to increase crop performance of eggplant when exposed to I biofortification programs, as well as to understand the reliability of the treatments through different growing years. Also, detecting the best combination of I doses and biostimulant application that could reduce I plant toxic effect, without reducing crop yield and quality, it would be of utmost importance in the current research.

2. Materials and methods

2.1. Cultivation conditions and experimental arrangement

The research was operated at the experimental field of the Department Agricultural, Food and Forestry Sciences of the University of Palermo. Seedlings of eggplant (*Solanum melongena* L.) "Birgah" F_1 hybrid (Fig. 1), were transplanted (2 plant m^{-2}) in open field on 2 May 2021 and 2022.

Plant mineral nutrition was supplied according to Di Miceli et al. (2023), considering the nutrient content of the soil. Climatic data (maximum and minimum temperatures and rainfall) were documented daily utilizing a data logger.

2.2. Treatments

For arbuscular mycorrhizal fungi (AM) inoculation, the protocol previously established was followed with slight modifications (Di Miceli et al., 2023). Briefly, for both years, inoculation with arbuscular mycorrhizal fungi (AM) was carried out using a solution containing spores of *Rhizophagus irregularis* (strain CMCCROC7, Bioplanet, Cesena, Italy), 400 spores per plant were administered by dipping the root system for 15 min, 24 h before transplanting. Inoculation was repeated 7 days after transplant by supplying 150 mL of solution per plant near to the root system, whereas non-inoculated plants received only water.

Protein hydrolysate (PH) application was achieved using Trainer (Hello Nature Italy SRL, Rivoli Veronese, Verona, Italy), a biostimulant

Fig. 1. Eggplant fruits of 'Birgah' F_1.

obtained throughout enzymatic hydrolysis of legumes, containing 31 % of plant amino acids and peptides, 35.5 % of organic matter, 5 % of organic nitrogen. PH-treatment started 10 days after transplanting and was applied throughout the cultivation cycle with an interval of 7 days. Eggplant plants were treated with a solution comprehending 2.5 mL L^{-1} of PH (manufactured recommended dose) or only with water (control plant), using 0.5 L m^{-2} of solution. Iodine biofortification was accomplished following the method described by Consentino et al. (2022, 2023) with slight modifications. Iodine was administered via potassium iodate (KIO$_3$), setting three doses: 0 (control), 300 or 600 mg L^{-1}. The treatments were supplied via foliar spray every 14 days, starting 10 days after transplant. For each I dose, 0.5 L m^{-2} of solution were supplied.

2.3. Mycorrhizal colonization, plant growth and yield

To assess the AM infection of the eggplant root system, the method of Giambalvo et al. (2023) was applied, analysing 3 plants per replicate. Mycorrhizal colonization was reported as percentage.

Data on plant height and number of leaves at 40 days after transplant (DAT) were collected. Furthermore, the first flowers emission was recorded, and the value was reported as DAT. Yield was weighed and separated in marketable and unmarketable until the end of the experiments (31st August 2021 and 31st August 2022). The marketable yield was determined by subtracting unmarketable fruit (bruising, morphological defects, unsuitable size) from the total yield.

2.4. Eggplant fruit dry matter content, anthocyanins, chlorogenic acid, antioxidant activity and iodine concentration

For fruit analysis, fruits from second to third harvest were collected. Five fully mature marketable fruits were casually picked from each replicate. The fruit dry matter content (reported as percentage) was assessed after drying an established weight of fresh eggplant sample (300 g) at 80 °C in a forced-air laboratory oven. The dried eggplant sample were listed and stored for further analysis.

The total anthocyanins content on fruit peel was determined following Mennella's method (Mennella et al., 2012). Shortly, 0.2 g of freeze-dried eggplant peel was diluted with 10 mL of methanol, then the anthocyanins content was determined via HPLC, using purified delphinidin-3-rutinoside as external standard.

Chlorogenic acid was assessed via a modified Stommel and Whitakers' procedure. To quantify chlorogenic acid concentration, eggplant samples were analysed by HPLC, using sesamol as internal standard and authentic chlorogenic acid as external standard setting the absorbance to 325 nm.

The antioxidant activity of fruit peel was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Sample of 2.5 g was ground and dissolved with methanol (80 %), then the samples were centrifuged. The absorbance of the mixture was measured at 517 nm. For calculating DPPH scavenging activity, the following formula was used (Molyneux, 2004):

$$AA \ [\%] = \frac{(A_0 - A_1)}{A_0} x100$$

AA: the antioxidant activity; A$_0$: the absorbance of the reference solution; A$_1$: the absorbance of the test solution.

To assess iodine eggplant fruit content, an extraction of the sample with tetramethylammonium hydroxide was performed, then via ICP-MS the concentration was determined.

2.5. Chlorophyll concentration and stress indicators

Chlorophyll content was estimated from six unharmed and fully expanded leaves randomly collected from each replicate. To determine chlorophyll a and chlorophyll *b*, the eggplant leaves samples were analysed using 80 % acetone method (Lichtenthaler, 1987) by

employing spectrophotometer (Perkin Elmer Lambda-EZ200) to document the absorbance data at wavelengths of 750 nm, 664 nm, 647 nm and 630 nm. Finally, after the readings, the content of Chl a, Chl b was calculated with the following equations:

$$Chlorophyll \ a = 12.21(A664) - 2.79(A647)$$

$$Chlorophyll \ b = 21.21(A647) - 5.10(A664)$$

Moreover, we calculated total chlorophyll (a+b) and the ratio between chlorophyll a and chlorophyll *b* (chlorophyll a/b).

The stomatal conductance was recorded in 2 undamaged and completely extended leaves per plant at 12:00 AM, between 3rd and 4th harvest.

For the estimation of proline levels, the colorimetric procedure suggested by Bates et al. (1973) with some modification by Toscano et al. (2016), was adopted. The technique is based on the response of proline with ninhydrin. Briefly, 1 g of fresh samples was blended with 5 mL of aqueous sulphosalicylic acid (3 %) and stirred, then 2 mL of this solution was homogenised with 2 mL of acetic acid and acid ninhydrin. The solution was exposed to a temperature of 100 °C for 1 h. After this time, the reaction was blocked by immersing the solution in ice and isolated with toluene. Finally, the absorbance was determined with the aid of a spectrophotometer.

The hydrogen peroxide (H$_2$O$_2$) content was assessed in accordance with the method reported by Velikova (2000). Briefly, 200 mg of sample was mixed with 2 mL of TCA solution at 0.1 % (W/V) and centrifuged (15,000 rpm for 15 min). Afterwards, 0.4 mL of potassium phosphate buffer (10 mmol L^{-1}) was added, and a calibration curve was generated to assess the amount of hydrogen peroxide, setting an absorbance of 390 nm.

The MDA concentration to evaluate lipid peroxidation was estimated agreeing to Heath and Packer (1968). In brief, 0.2 g of tissue was homogenized with 5 mL of trichloro-acetic acid (5 % w/v) and centrifuged (12,000 rpm for 20 min). after that, 3 mL of supernatant was mixed with 0.5 % (w/v) TBA in 20 % (w/v) TCA and heated at 90 °C for 30 min. After cooling, the mixture was centrifuged at 7500 rpm. Absorbance was recorded at 532 and 600 nm.

2.6. Experimental design and statistics

An experiment enclosing four biostimulant treatments (control, PH, AM or PH+AM) and three I dosages (0, 300 or 600 mg/L) was set-up in a split-plot experimental design, rendering 12 treatments. Each treatment was replicated three times, containing 30 plants (10 per replicate), for a total of 360 eggplant plants. The trial was performed for two succeeding years (2021 and 2022) and data between the years were compared.

The entire dataset was tested for the ANOVA assumption, using Shapiro-Wilk and Levene tests. Statistical analysis was performed using SPSS software version 28.0, using the general linear model (GLM). All agronomic and qualitative data obtained were subjected to a three-way ANOVA set biostimulant, I and year as main factors. The mean values were separated via Tukey's HSD test at 0.05 significance level. Data expressed as percentage were exposed to arcsin conversion before ANOVA.

3. Results

3.1. Climatic data

During 2021 and 2022 cultivation period (from 2nd May to 31st August) rainfall was 79.11 and 80.56 mm, respectively.

Average maximum temperatures were higher in 2022 than in 2021especially in May, June, and July (Fig. 2). Moreover, the highest minimum temperatures, mainly in May, July and August, were recorded in the second year of cultivation (2022).
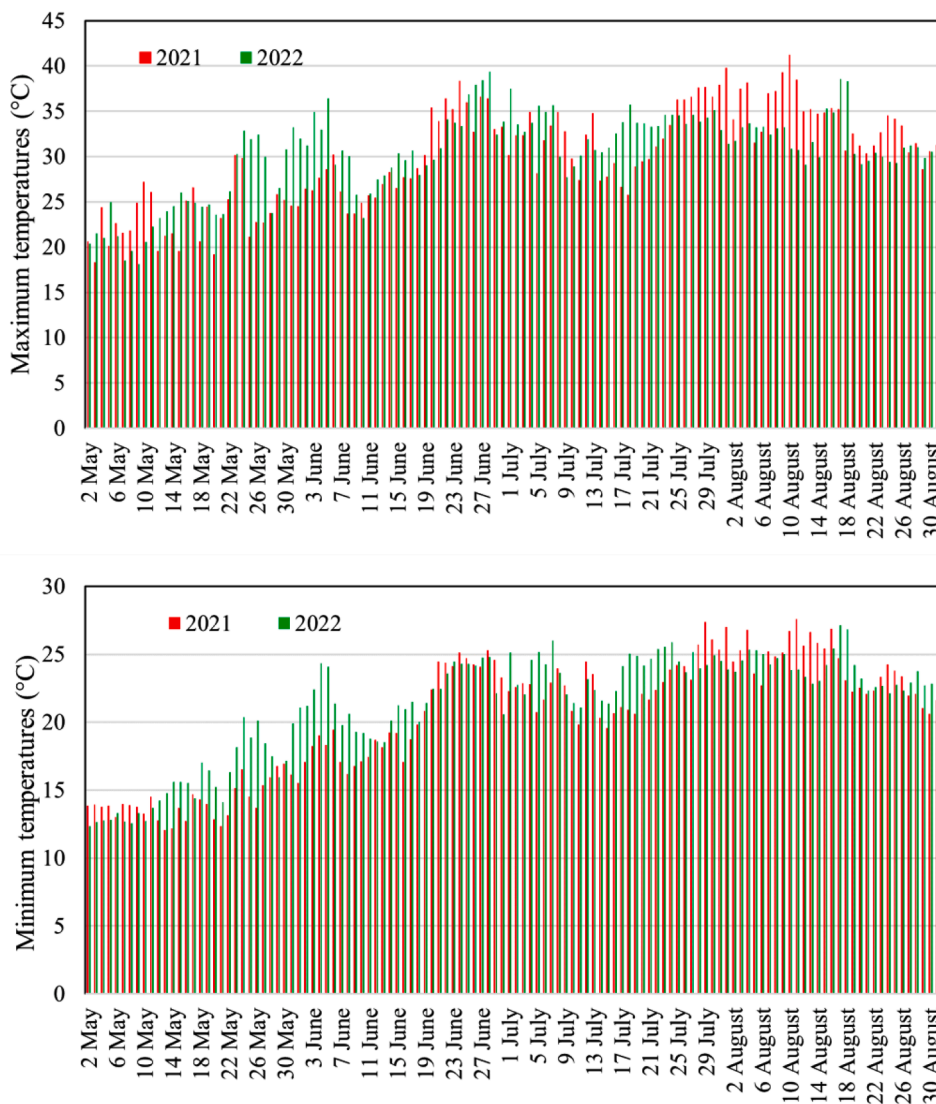
Fig. 2. Maximum (a) and minimum (b) temperatures recorded from 2nd May to 31st August at the experimental field.

3.2. ANOVA output for all recorded parameters

All ANOVA output is presented in Table S1. Statistical analysis revealed that I dosages significantly affected all recorded parameters except for mycorrhizal colonization, whereas biostimulants meaningfully modulated all recorded parameters. Interestingly, the year significantly modulated all parameters apart from mycorrhizal colonization, fruit dry matter, I, chlorophyll a/b ratio, proline, H_2O_2 and malondialdehyde. Moreover, our analysis revealed a significant interaction between the treatments. All parameters were affected by the

Table 1

Impact of iodine and biostimulants on plant height 40 days after transplant, number of leaves, first flower emission, total yield, marketable yield and mycorrhizal colonization of eggplant plant.

Treatments	Plant height 40 DAT (cm)		No. Leaves 40 DAT		First flower emission (DAT)		Total yield (kg plant^{-1})		Marketable yield (kg plant^{-1})		Mycorrhizal colonization (%)	
Iodine (mg/L)												
0	50.5	a	22.4	a	52.9	a	4.2	a	3.9	a	38.2	a
300	40.1	b	21.3	b	52.6	a	4.2	a	3.8	b	38.6	a
600	35.2	c	20.7	c	49.7	b	3.2	b	2.9	c	38.9	a
Biostimulant												
Control	36.6	d	16.5	d	50.4	c	3.4	d	3.0	d	4.0	b
PH	43.0	b	22.3	b	53.8	a	4.0	b	3.6	b	4.5	b
AM	41.4	c	20.8	c	51.0	bc	3.7	c	3.5	c	70.6	a
PH + AM	46.8	a	26.2	a	51.7	b	4.4	a	4.1	a	70.0	a
Year												
1	41.1	b	20.2	b	52.3	a	3.8	b	3.5	b	37.9	a
2	42.8	a	22.7	a	51.2	b	3.9	a	3.6	a	39.2	a

Means having different letters are dissimilar according to the Tukey HSD test. PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants; Year 1: 2021; Year 2: 2022.

interaction I × biostimulants except for number of leaves, mycorrhizal colonization, chlorogenic acid and chlorophyll a. The interaction I × year significantly affected first flower emission, total anthocyanins, and total antioxidant activity of peel, whereas the interaction biostimulant × year only affected total anthocyanins. Finally, our data revealed that all three main factors (iodine, biostimulant and year) significantly interacted each other modulating total anthocyanins and total antioxidant activity of peel.

3.3. Eggplant growth, yield, and mycorrhizal colonization

Regardless of biostimulant and year, when the I dosages increased, plant height, number of leaves and marketable yield values decreased (Table 1).

The highest first flower emission and total yield values were recorded in control plants and in plants treated with 300 mg L^{-1} of I (in form of KIO$_3$). Whereas, ANOVA did not display a significant impact of the I treatment on mycorrhizal colonization. Regarding the biostimulant treatment, plants subjected to a combined application of PH and AM revealed the highest plant height values (+27.9 % compared to control), followed by those treated with PH (+17.5 % compared to control); the lowest values were logged in control plants (36.6 cm). Data on number of leaves, total yield and marketable yield followed the trend described for plant height (Table 1). The highest average value of first flower emission was recorded in PH-treated plants (+6.7 % compared to control), followed by that recorded on plants treated with both biostimulants (+2.6 %); the lowest value was observed in control plants (50.4 DAT). The mycorrhizal colonization percentage was increased by AM inoculation; indeed, plants treated with AM or PH+AM had the highest colonization percentage (70.6 % and 70.0 %, respectively), while control and PH-treated plants (those non inoculated) revealed the lowest ones (4.0 % and 4.5 %, respectively) (Table 1). Our results also showed that the year had a significant effect on all parameters presented in Table 1. Plants cultivated in year 2 had higher height (+4.13 %), number of leaves (+12.4 %), total yield (+2.6 %) and marketable yield (2.8 %) than plants cultivated in year 1, whereas an opposite tendency was shown for first flower emission (-2.14 %) (Table 1). Plants height 40 DAT was also influenced by the interaction I × biostimulant (Fig. 3a).

Overall, regardless of the I dosages, biostimulants significantly enhanced plant height compared to the control (Fig. 3a). However, the highest values were measured in non-biofortified plants treated with both biostimulants (+31.4 % compared to non-treated plants), followed by those recorded in plants not exposed to I and treated with PH (+ 23.8 % compared to non-treated plants) (Fig. 3a). The lowest values were achieved by control plants treated with 600 mg/L of I (31.1 cm).

Results also revealed that first flower emission value was modulated by the interaction between I and biostimulant (Fig. 3b). As showed in Fig. 1b, PH-treated plants non-enriched or supplied with 300 mg/L of I had the highest values (56.0 and 55.5 DAT, respectively), followed by those biofortified with 300 mg/L of I and treated with PH+AM (52.2 DAT) (Fig. 3b). The lowest average value in terms of first flower emission was recorded in control plants treated with 600 mg/L of I (48.7 DAT). Moreover, I and year significantly interacted in modulating first flower emission (Fig. 3c). When 0 and 300 mg/L of I were applied, plants cultivated in year 1 revealed higher values than those grown in year 2 (53.7 *vs* 52.1 and 53.5 *vs* 51.7 DAT, respectively for 0 and 300 mg/L of I). Plants grown in the first and second year and exposed to 600 mg/L revealed the lowest values (49.6 and 49.8 DAT) (Fig. 3c).

Data showed that I and biostimulant significantly influenced total yield and marketable yield (Fig. 4a and b).

Plants treated with both biostimulants and supplied with 0 or 300 mg/L of I had the highest total yield, achieving 4.8 and 4.7 kg plant^{-1}, respectively; these values were followed by those recorded in non-biofortified plants treated with PH (4.4 kg plant^{-1}). Control plants enriched with 600 mg/L of I had the lowest total yield (2.7 kg plant^{-1}) (Fig. 4a). For marketable yield, the 0 × PH+AM and 300 × PH+AM combinations peaked with 4.5 and 4.4 kg plant^{-1}, respectively; the lowest yields was found in control plants treated with 600 mg/L of I (2.4 kg plant^{-1}) (Fig. 4b).

3.4. Qualitative traits of eggplant fruits and iodine concentration

When mediated over biostimulant and year, dry matter percentage of eggplant fruits decreased as the dose of I increased (Table 2).

The highest total anthocyanins concentration was recorded in fruits from plants treated with the highest I dosage (8468.1 mg 100 g^{-1} DW), followed by those from plots supplied with 300 mg/L (8347.3 mg 100 g^{-1} DW); control plants had the lowest value (8055.9 mg 100 g^{-1} DW). The trend described for total anthocyanins was similar to that recorded for chlorogenic acid, total antioxidant activity and I (Table 2). Disregarding of I supply and year, the highest fruit dry matter was found in plants treated with PH and AM (7.1 %), followed by that recorded in the plants inoculated with AM (6.9 %); the lowest value was recorded in fruits from control plants (6.3 %). Plants treated with PH or with PH+AM had the highest fruit anthocyanin concentration (+2.3 % and +2.7 % compared to control, respectively), whereas control plants revealed the lowest fruit anthocyanins concentration (8161.1 mg 100 g^{-1} DW) (Table 2). The chlorogenic acid concentration peaked in plants treated with both biostimulants, followed by that recorded in plants exposed to PH or AM. The antioxidant activity of peel was significantly reduced by biostimulant application; indeed, the highest value was found in fruits from control plants, followed by that from plants inoculated with AM. The lowest value was recorded in fruits from plants treated with both biostimulants (Table 2). Iodine concentration peaked in fruits from plants treated with PH+AM (101.1 mg kg^{-1} DW), followed by that from fruits of PH-treated plants (92.4 mg kg^{-1} DW) and AM-inoculated plants (80.5 mg kg^{-1} DW). Control plants revealed the lowest I value (75.6 mg kg^{-1} DW) (Table 2). Non-considering I and biostimulant, our data revealed that fruits from plants cultivated in year 2 had a higher total anthocyanin and chlorogenic acid concentration than those from eggplants cultivated in year 1, whereas for total antioxidant activity of peel an opposite trend was recorded (Table 2).

Fruit dry matter was modulated by the I × biostimulant interaction (Fig. 5a).

In general, biostimulants (alone or combined) significantly increased the percentage of fruit dry matter compared to the control, however this result was only recorded in biofortified plants (Fig. 5a). In fact, in non-biofortified plots, the plants treated with PH did not show statistically significant differences when compared with the control plants. The highest fruit dry matter values were documented in plants treated with the combinations 0 × AM, 0 × PH+AM and 300 × PH+AM, whereas the lowest was documented in 600 × control plots (Fig. 5a).

Total anthocyanins values were influenced by the interaction I × biostimulant × year (Fig. 5b). Data revealed that anthocyanins concentration in fruits was enhanced by I dosage and biostimulant with the peak in fruits from plants cultivated in year 2 treated with the highest I dosage and supplied with both biostimulants (8545.1 mg 100 g^{-1} DW), followed by those collected from plants cultivated in year 1 and treated with 600 mg L^{-1} of I and by those cultivated in year 2, treated with 600 × PH or 600 × AM (Fig. 5b). The lowest value was recorded in plants non-exposed neither to I, nor to biostimulants.

In both experimental years, fruits from control plants treated with 600 mg L^{-1} of I had the highest total antioxidant activity of peel, followed by those from mycorrhized plants cultivated in year 1 and treated with 600 mg L^{-1} of I (Fig. 5c). The lowest values were recorded in non-biostimulated plants cultivated in year 1 (6.3 %DPPH) and 2 (6.3 % DPPH) and treated with 0 mg L^{-1} of I (Fig. 5c).

Iodine concentration in eggplant fruits was significantly affected by the interaction I × biostimulants (Fig. 6).

Results showed that the biostimulants significantly enhanced the I concentration in biofortified plots. Plants biofortified with the highest I concentration and exposed to both biostimulants accumulated the
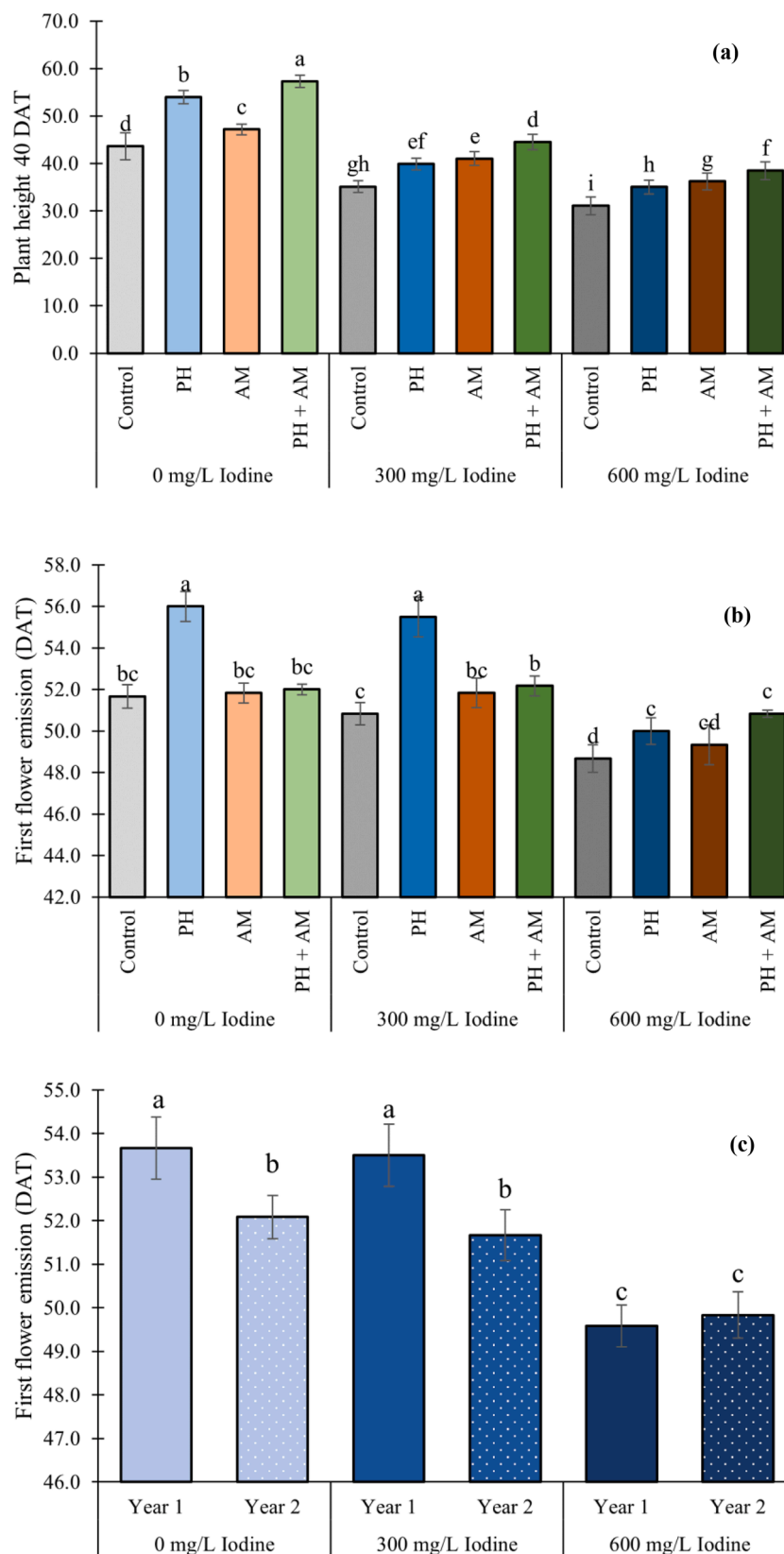
Fig. 3. Impact of iodine and biostimulants on plant height 40 days after transplant (a) and first flower emission (b) of eggplant; effect of iodine and year on eggplant first flower emission (c). Means having different letters are dissimilar according to the Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants; Year 1: 2021; Year 2: 2022.
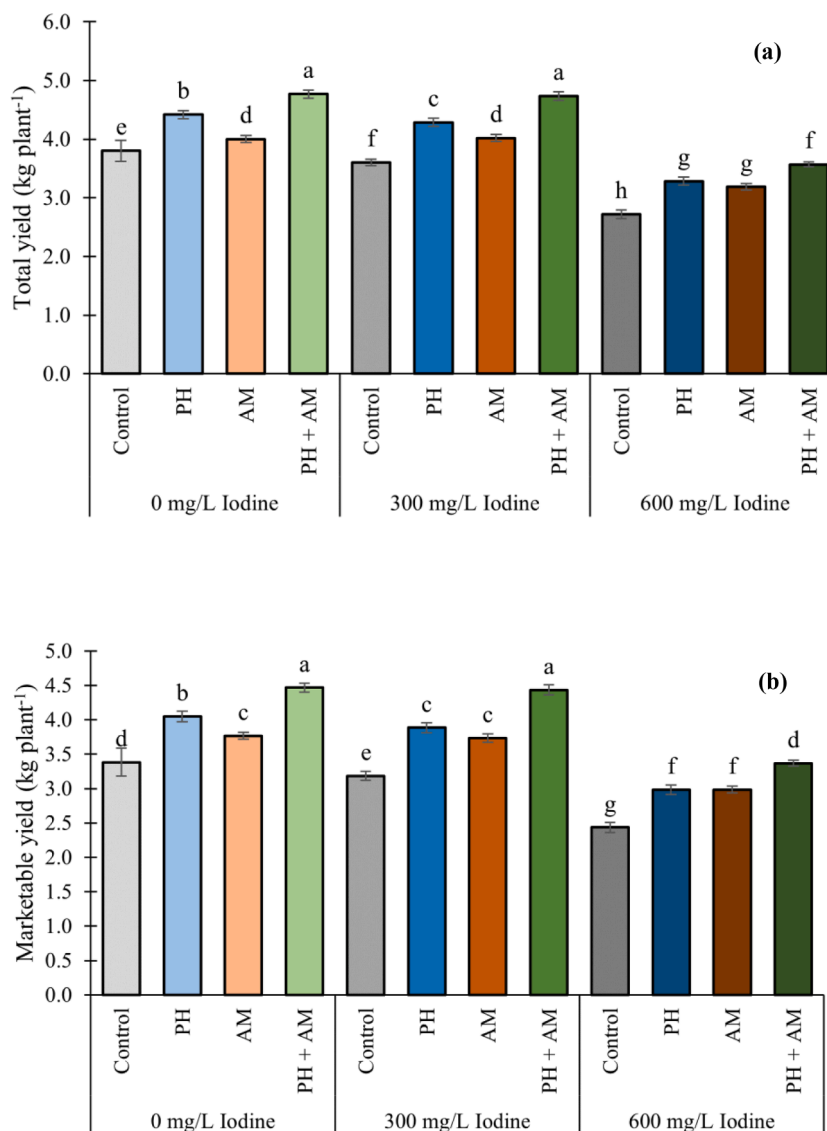
Fig. 4. Impact of iodine and biostimulants on total yield (a) and marketable yield (b) of eggplant plants. Means having different letters are dissimilar according to the Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants.

Table 2
Impact of iodine and biostimulants on fruit dry matter, total anthocyanins, chlorogenic acid, total antioxidant activity of peel and iodine of eggplant fruits.

Treatments	Fruit dry matter (%)		Total anthocyanins (mg 100 g^{-1} DW)		Chlorogenic acid (mg 100 g^{-1} DW)		Total antioxidant activity of peel (% DPPH)		Iodine (mg kg^{-1} DW)	
Iodine (mg/L)										
0	7.1	a	8055.9	c	779.1	c	7.4	c	0.1	c
300	6.9	b	8347.3	b	853.2	b	8.2	b	116.1	b
600	6.2	c	8468.1	a	965.8	a	10.6	a	146.0	a
Biostimulant										
Control	6.3	d	8161.1	c	848.9	c	9.3	a	75.6	d
PH	6.7	c	8346.8	a	863.1	b	8.5	c	92.4	b
AM	6.9	b	8272.9	b	864.3	b	9.0	b	80.5	c
PH + AM	7.1	a	8381.1	a	887.9	a	8.2	d	101.1	a
Year										
1	6.8	a	8268.6	b	858.5	b	8.8	a	86.99	a
2	6.8	a	8312.3	a	873.6	a	8.7	b	87.84	a

Means having different letters are dissimilar according to the Tukey HSD test. PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants; Year 1: 2021; Year 2: 2022.
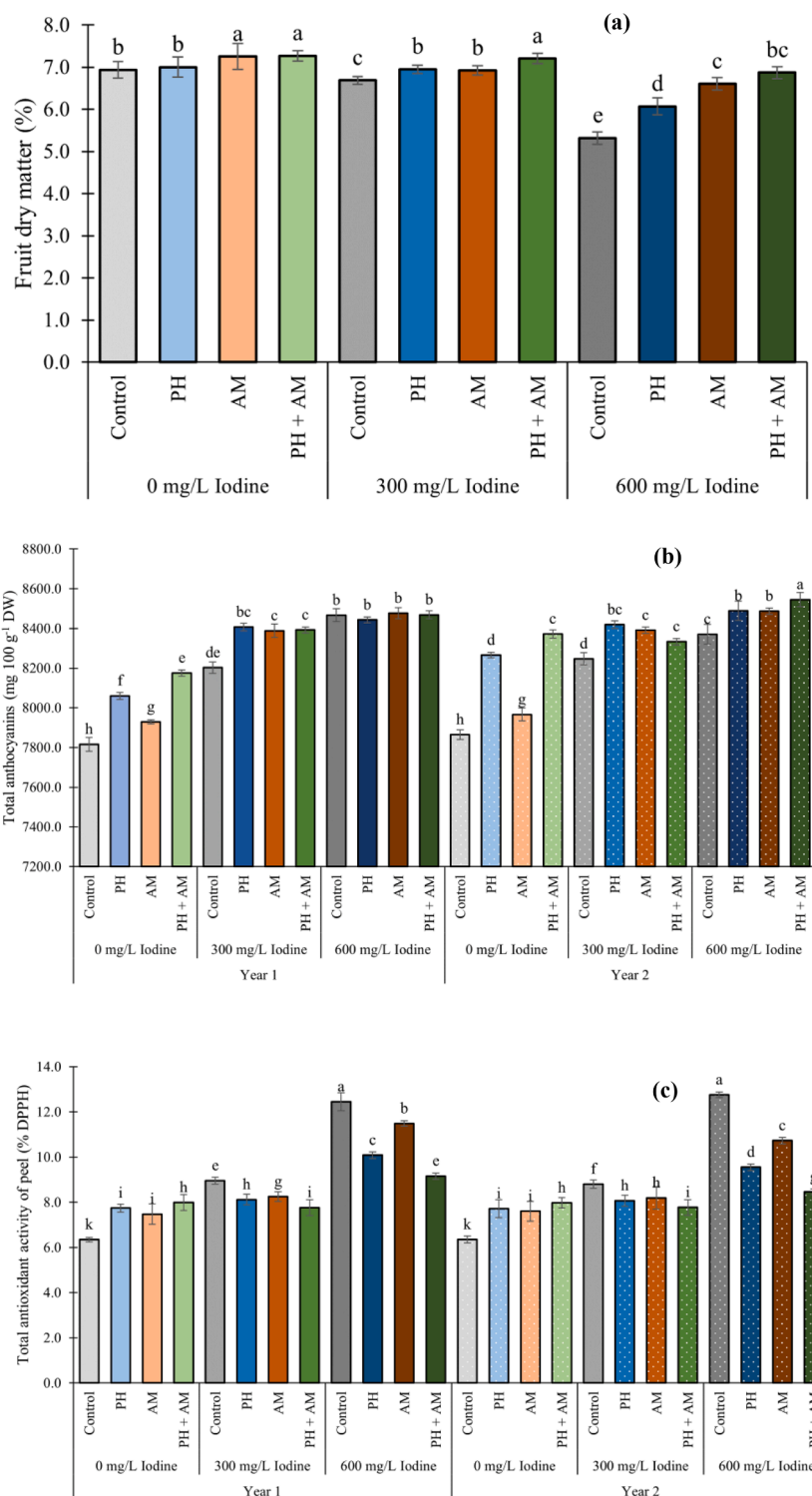
Fig. 5. Impact of iodine and biostimulants on eggplant fruit dry matter (a); effect of iodine, biostimulant and year on eggplant total anthocyanins (b) and total antioxidant activity of peel (c). Means having different letters are dissimilar according to Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants; Year 1: 2021; Year 2: 2022.

highest amount of I in the fruits (167.52 mg kg^{-1} DW), followed by those from 600 × PH plots (155.73 mg kg^{-1} DW) (Fig. 6). Fruits from non-biofortified plants had the lowest I concentration (average 0.12 mg kg^{-1} DW).

3.5. Chlorophylls and stress indicators

Regardless of biostimulant and year, chlorophyll a decreased as I dosage increased (Table 3).

Results on chlorophyll *b*, chlorophyll a+b and stomatal conductance followed the trend designated for chlorophyll a (Table 3). However, chlorophyll a/b ratio peaked in plants treated with 600 mg L^{-1} of I,
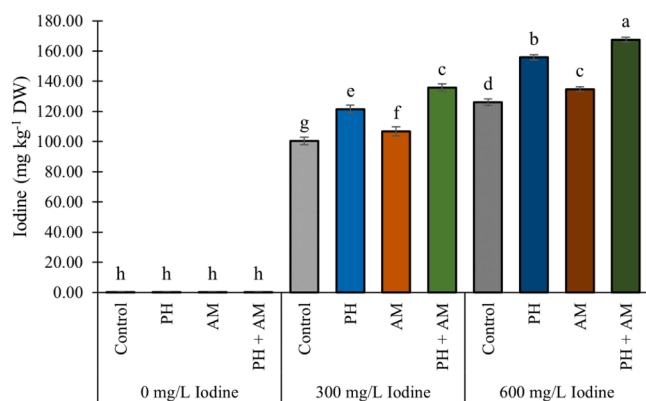
Fig. 6. Impact of iodine and biostimulants on iodine concentration of eggplant fruits. Means having different letters are dissimilar according to Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants.

whereas it was at the lowest level in control plants. Data on proline, H_2O_2 and malondialdehyde (MDA) concentration overlapped with those already described for chlorophyll a/b ratio (Table 3). When mediated over I and year, plants treated with PH+AM revealed the highest chlorophyll a values (+58.9 % compared to the control), followed by those treated with PH (+43.8 % compared to the control), which in turn revealed higher values than those treated with AM (+17.1 % compared to the control) (Table 3). Findings on chlorophyll *b*, chlorophyll a+b and stomatal conductance matched with those described for chlorophyll a (Table 3). The highest chlorophyll a/b ratio was recorded in plants inoculated with AM, followed by that recorded in control plants and in those treated with both biostimulants. Plants treated with PH did not significantly differ neither from AM-treated plants, nor from control or PH-treated plants (Table 3). Plants treated with PH+AM revealed the highest proline concentration, followed by those treated with PH or AM alone. The highest H_2O_2 values were recorded in control plants, followed by those recorded in plants treated with both biostimulants (PH+AM); the lowest values were found in plants inoculated with AM (Table 3). Results on MDA concentration followed the trend described for proline (Table 3). Non-considering I dosages and biostimulants, plants grown in year 2 had higher values in terms of chlorophyll a, chlorophyll *b*, chlorophyll a+b and stomatal conductance than those cultivated in the year 1 (Table 3).

Chlorophyll a, chlorophyll *b* and chlorophyll a+b values were influenced by the interaction between I and biostimulant (Fig. 7a–c).

For these three parameters, the highest values were noted in non-biofortified plants treated with PH+AM, followed by those exposed to

the combination 0 × PH (Fig. 7a–c). However, the lowest values for chlorophyll a and chlorophyll a+b were documented in control plants treated with 600 mg L^{-1} of I. For chlorophyll *b* the lowest concentrations were found in plants treated with the 600 × control and 600 × AM combinations (Fig. 7b). The highest chlorophyll a/b ratio was collected in AM inoculated plants fertigated with 600 mg L^{-1} of I, followed by those enriched with the same I dose and treated with PH (Fig. 7d). The lowest values were detected in non-biofortified plants treated with PH or PH+AM.

Stomatal conductance was influenced by the interaction between I and biostimulant (Fig. 8).

In general, regardless of the I dosage, biostimulants significantly enhanced stomatal conductance of eggplant compared to the control. Plants from non-biofortified plots treated with PH+AM revealed the highest values, followed by those from the 0 × PH and 300 × PH+AM combinations. Control plants, enriched with 600 mg L^{-1} of I had the lowest stomatal conductance (Fig. 8).

Proline was also affected by the interaction I × biostimulant (Fig. 9a).

Biostimulants enhanced proline concentration at any I dosages (Fig. 9a). However, the highest concentration was assessed in plants supplied with the highest I dosage and treated with both biostimulants, while the lowest one was detected in plants grown without biostimulants and I supply.

For H_2O_2, ANOVA underlined a significant effect of the interaction between I and biostimulant (Fig. 9b). In particular, the highest amounts were observed in control plants biofortified with 600 mg L^{-1} of I, followed by those in plants treated with the same I dosage and treated with PH or PH+AM (Fig. 9b). The lowest values were found in untreated plants (not biostimulated and not biofortified).

Malondialdehyde concentration was influenced by the interaction between I and biostimulant (Fig. 9c). The MDA peak was recorded in plants treated with both biostimulants and supplied with the highest I dose (+74.8 % compared to non-treated plants), while the lowest value was recorded in non-treated plants.

4. Discussion

With a view to produce micronutrient-rich crops, agronomic biofortification plans often provide the supply of high trace element dosages. However, such procedure may cause plant phytotoxicity and, concomitantly, biofortification program failure. In this regard, it merits consideration that the application of pioneering sustainable means for increasing crop tolerance to abiotic distresses could be of paramount concern. Therefore, the present experiment targeted to estimate the impact of two biostimulants ('Trainer' protein hydrolysate and *Rhizophagus irregularis* arbuscular mycorrhizal fungi) on eggplant plants treated with three I dosages. In both experimental years, the mycorrhizal

Table 3

Impact of iodine and biostimulants on chlorophyll (a, b, a+b and a/b), stomatal conductance, proline, H_2O_2 and malondialdehyde concentrations of eggplant plants.

Treatments	Chlorophyll a (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Chlorophyll a+b (mg g^{-1} FW)		Chlorophyll a/b ratio		Stomatal conductance (mmol m^{-2} s^{-1})		Proline (μmol g^{-1} FW)		H_2O_2 (nmol g^{-1} FW)		MDA (μmol g^{-1} FW)	
Iodine (mg/L)																
0	22.5	a	9.7	a	32.3	a	2.37	c	351.0	a	9.19	c	38.54	c	5.54	c
300	19.4	b	6.9	b	26.3	b	2.80	b	303.5	b	18.86	b	49.15	b	6.63	b
600	14.9	c	4.6	c	19.5	c	3.33	a	273.9	c	20.39	a	63.84	a	7.27	a
Biostimulant																
Control	14.6	d	5.3	d	19.9	d	2.80	b	258.6	d	13.69	c	53.94	a	6.13	c
PH	21.0	b	7.9	b	28.9	b	2.83	ab	322.9	b	16.70	b	49.69	c	6.35	b
AM	17.1	c	6.0	c	23.1	c	3.00	a	284.0	c	16.51	b	47.81	d	6.34	b
PH + AM	23.2	a	9.2	a	32.4	a	2.65	b	372.4	a	17.69	a	50.61	b	7.10	a
Year																
1	18.7	b	6.9	b	25.6	b	2.8	a	303.6	b	16.09	a	50.65	a	6.45	a
2	19.2	a	7.2	a	26.5	a	2.8	a	315.3	a	16.20	a	50.37	a	6.51	a

Means having different letters are dissimilar according to the Tukey HSD test. PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants; Year 1: 2021; Year 2: 2022.
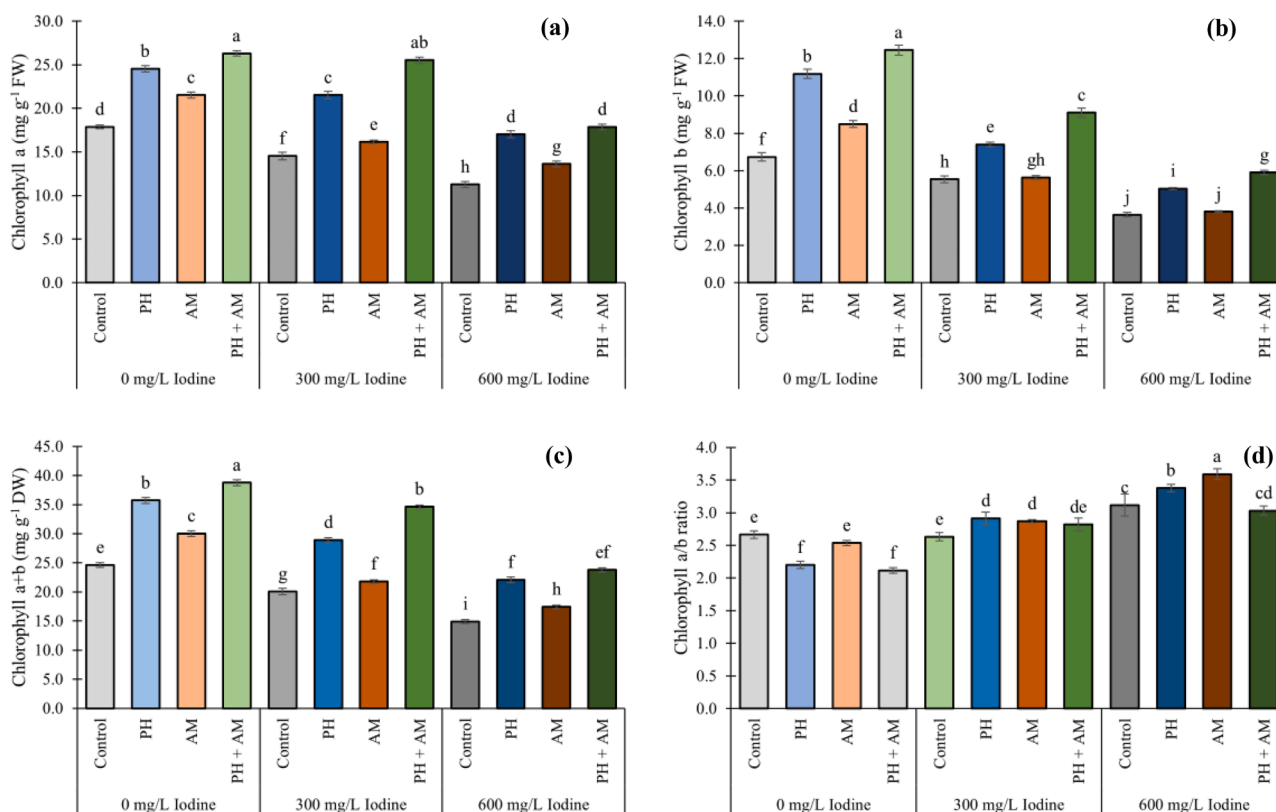
Fig. 7. Impact of iodine and biostimulants on chlorophyll a (a), b (b), a+b (c) and a/b (d) of eggplant plants. Means having different letters are dissimilar according to Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants.
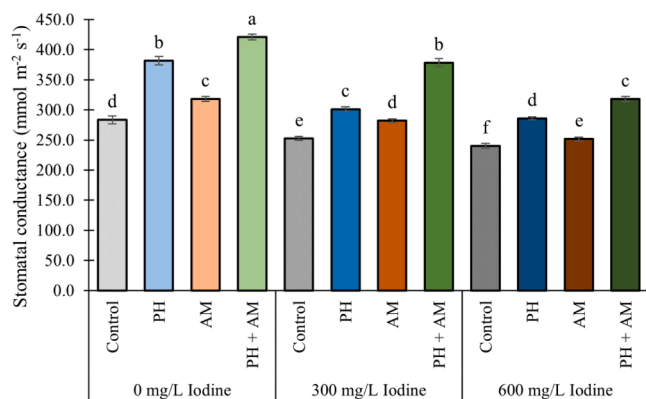

Fig. 8. Impact of iodine and biostimulants on stomatal conductance eggplant plants. Means having different letters are dissimilar according to Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants.

colonization percentage was higher in plants supplied with AM or with PH+AM. The colonization percentage reached 70.6 % and 70.0 % in AM and PH+AM treated plants, respectively, whereas I did not influence the colonisation rate of the roots. There results could be connected to the fact that I was distributed via foliar spray, non-influencing the nutrient solution parameters (e.g. electrical conductivity) and, consequently, the AM growth environment. Moreover, in line with our outcomes, there are no evidence of the I interference with root mycorrhizal symbiosis. The study also showed that the year did not statistically influence root colonisation. This result indicates that the temperatures fluctuated within a range suitable for the AM activity. Interestingly, the treatments

PH+AM did not significantly differ from the AM treatment, thus, we can declare that PH application did not affect mycorrhizal colonization percentage. Briefly, we can assert that inoculation with AM was successful (about 70 % colonization vs 4 %) and that the other experimental factors did not influence this parameter.

Plant height and number of leaves were decreased by I dosages. As stated by Zhang et al. (2023), I is not contemplated as a necessary trace element for plants since there are no evidence of its role on plant metabolism. Even though low dosages of I could have helpful impacts on plant growth (Kiferle et al., 2013; Incrocci et al., 2019), it was reported that high dosages of I could interfere with plant growth and development, causing physiological disorders (Welch and Schuman, 2011; Zhang et al., 2023). Consequently, the decrease in growth traits could be ascribed to the high I dosages tested (300 and 600 mg L^{-1}). Eggplant growth traits were enhanced by biostimulant application. As testified by various authors (Ertani et al., 2014; Zuluaga et al., 2023), PH stimulate plant primary metabolism, influencing various metabolic pathways. At this regard, the primary effect of PH is due to amino acids, which represent the principal way of transport for organic nitrogen for plants and can also be employed for protein synthesis (Colla et al., 2015). Regarding AM, the positive consequence registered on plant growth is connected to the increase of nutrients absorption and translocation, as well as to the expanded root system of inoculated plants (Rouphael et al., 2015; Sabatino et al., 2020). Remarkably, plants treated with both biostimulants showed superior growth traits compared to those treated with PH or AM individually. Thus, we can assume that PH and AM synergistically interacted in boosting plant growth traits. Our study also outlined that plants cultivated in 2022 (year 2) had higher growth parameters than those grown in 2021 (year 1). The effect of the year on height and number of leaves can be related to the higher temperatures registered in year 2. Indeed, we know that eggplant is a macrothermal species, therefore it takes advantage of high temperatures. Moreover, it
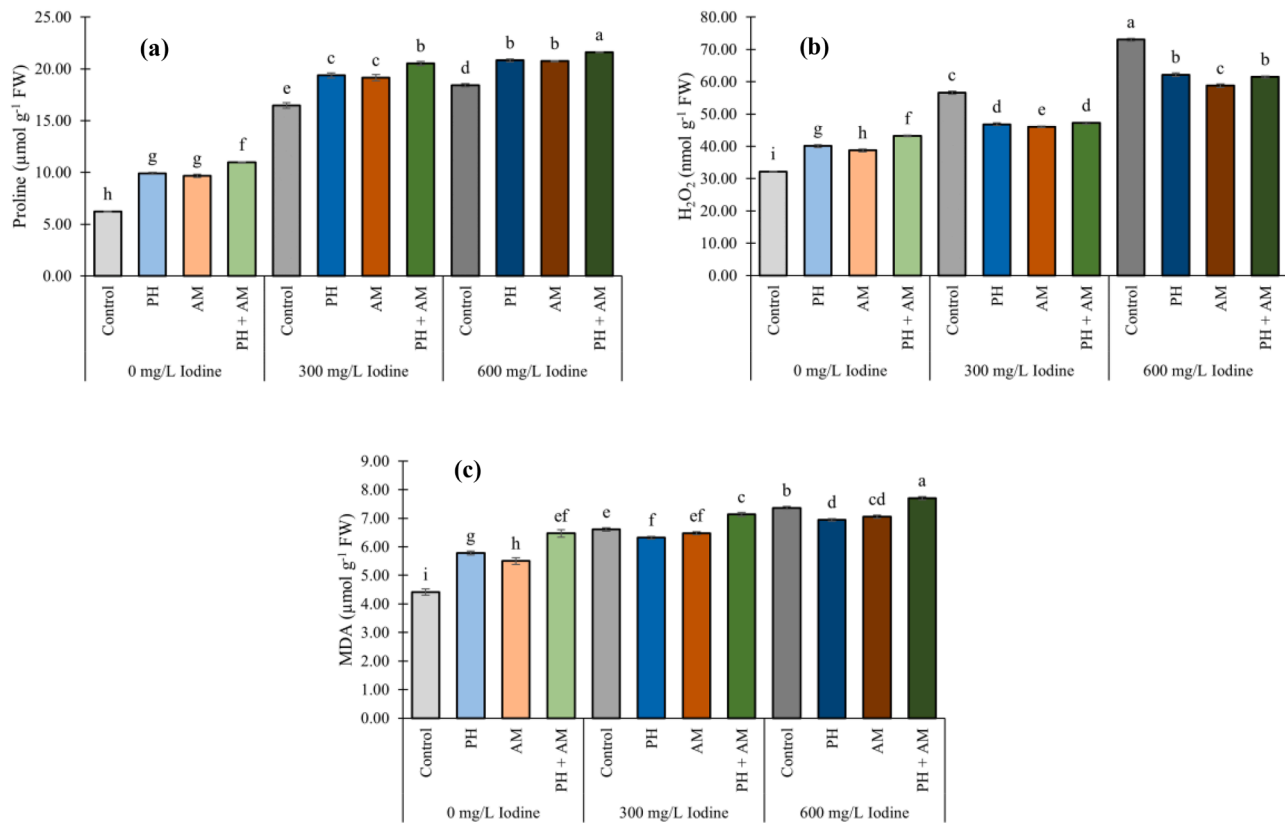
Fig. 9. Impact of iodine and biostimulants on proline (a), H$_2$O$_2$ (b) and malondialdehyde (c) of eggplant plants. Means having different letters are dissimilar according to Tukey HSD test. Results are presented as mean ± SE. Control: non-treated; PH: protein hydrolysates; AM: arbuscular mycorrhizal fungi; PH+AM: mutual application of both biostimulants.

was recorded that all I dosage biostimulants enhanced plant height compared to the control. This result is very noteworthy, since it seems that both biostimulants had a buffer effect on I toxicity. The beneficial effect of PH can be related to an increase of plant defence mechanisms against abiotic stresses prompted by amino acids and peptides which stimulate proline biosynthesis, which in turn has positive effect on plant abiotic stress tolerance (Malécange et al., 2023). On the other hand, the AM effect on plants could be related to an improvement of ROS scavenging enzymes, which led to the reduction of I oxidative damage (Zou et al., 2021). Even when in combination with I, the two biostimulants synergistically interacted in increasing plant height, demonstrating that they can cooperate to alleviate I toxic effects.

Since stressed plants bloom earlier than those not stressed (Takeno, 2016) and considering that the application of high doses of I is a plant stress factor (Kiferle et al., 2019), the results on first flower emission are attributable to a physiological response of the plant to I supply. Whereas, the delayed flowering of biostimulated plants could be the effect of an increased vigour. Indeed, as previously showed, biostimulated plants had high growth traits. Since biostimulants elicited plant vegetative activity and considering the plant endogenous homeostasis between vegetative versus reproductive phase, we may hypothesize that the recorded flowering delay of the biostimulated plants is related to the higher plant vigour. Regarding the year, data showed that plants grown in the second year (2022) had a lower first flower emission values than those grown in the first year (2021). This outcome can be interpreted as the response of plants to different environmental situation. In fact, the second year of cultivation was warmer than the first one, enhancing eggplant plant vigour traits. Moreover, for first flower emission, we recorded a significant interaction between I and biostimulants. According to Takeno et al. (2016), these outcomes clearly showed the reducing effect of biostimulants against stress caused by I. Our study also

pointed out that first flower emission value was affected by the interaction between I and year. Our results indicated that the more favourable environmental conditions in year 2 induced earlier flowering in combination with 0 or 300 mg L^{-1} of I application. However, when plants were treated with 600 mg L^{-1}, regardless of the year, first flower emission drastically decreased suggesting that the temperature effect was outweighed by the highest I dosage.

Total and marketable yield were reduced by the highest I dosages (300 or 600 mg L^{-1}). As stated by Kiferle et al. (2021), I is mainly stored in the chloroplasts, thus, it seems reasonable that the reduction in terms of yield could be due to a negative effect on the plant photosynthetic system. In our study, biostimulants elicited yield traits. Rouphael and Colla (2020) reported the positive effects of both biostimulants in increasing plants growth traits and, consequently, photosynthesis rate. Remarkably, the highest total and marketable yield were logged in plants treated with both biostimulants, indicating that they synergistically interact. The higher total and marketable yield detected in the second experimental year could be connected to the beneficial higher temperatures recorded. Our experiment also underlined a significant interaction between I and biostimulant for total and marketable yield. We believe that the I negative effects on total and marketable yield - observed in control plants treated with I - were mitigated by the application of biostimulants, especially in the combination PH+AM.

Dry matter is strictly related to photosynthesis and considering that I can negatively affect chlorophyll concentrations in plants (Kiferle et al., 2021), we may assume that the fruit dry matter content reduction observed in our study was a result of I effect on chlorophyll. Conversely, the positive effect of PH on dry matter content can be linked to its effect on nitrogen metabolism. Indeed, as evidenced by Colla et al. (2015), amino acids included in PH play a significant role in boosting N accumulation and transport. The positive effect of AM inoculation on dry

matter content can be related to an increased uptake and nutrients translocation (Xie et al., 2022). In this scenario, it is of particular interest the interaction between I and biostimulants when jointly supplied (PH+AM); indeed, in this case the mutual application of both biostimulants mitigated the negative effect of I on dry matter content better than PH or AM alone. This defensive effect could be attributed to the biostimulant ability for triggering the biosynthesis of compounds with antioxidant activity (Santander et al., 2020; Zuluaga et al., 2023; Soussani et al., 2023).

Iodine application increased total anthocyanins concentration in fruits. This could be described as a plant defence mechanism against the oxidative stress. Indeed, stressed plants biosynthetise flavonoids with high antioxidant ability, like anthocyanins (Li and Ahammed, 2023). Data on anthocyanins are also in line with those of Di Mola et al. (2020), who found an increase of secondary metabolism activity in lamb's lettuce and baby spinach treated with PH. Moreover, the effect of AM on anthocyanins were documented by Chiomento et al. (2019) and Parada et al. (2019) on strawberry. Fascinatingly, anthocyanins values in plants treated with PH or PH+AM did not statistically differ. In this respect, we believe that the predominant effect on anthocyanins was provided by the application of PH rather than by AM. Consequently, in this case, there was no synergy between the biostimulants. Plants cultivated in the second year revealed the highest fruit anthocyanins concentration. Since higher temperature promotes anthocyanin biosynthesis, our results could be related to the higher temperatures logged throughout the second year. In our research, all the experimental factors significantly interacted in modulating anthocyanins concentration. In general, biostimulants and I augmented the anthocyanin concentration in eggplant fruit, with a slight increase in the second year due to the higher temperatures.

Outcome on chlorogenic acid ties well with the results of Pardossi et al. (2015), who showed that polyphenol increase is a result of I distress as a plant defence mechanism activation. Fruits from biostimulated plots had higher chlorogenic acid values than those from control ones. There are reports concerning the positive effect of PH on polyphenols, such as chlorogenic acid (Caruso et al., 2019; Giordano et al., 2022). Furthermore, as reported by Avio et al. (2017), AM inoculation may interact with plant secondary metabolism, generating an increased biosynthesis of phenolic compounds. Interestingly, the joint application of PH and AM gave the highest chlorogenic acid concentration in eggplant fruits. Consequently, we can assume that both biostimulants are compatible and synergetic to increment this parameter. Moreover, data underlined a significant improve in chlorogenic acid concentration in fruits from plants cultivated in year 2. As explained by Helyes et al. (2015), we may assume that the increased presence of chlorogenic acid was the plant's response to environmental conditions.

Outcomes on total antioxidant activity are in line with those of Incrocci et al. (2019) and Kiferle et al. (2019), who stated that the antioxidant capacity of plants is positively correlated with the amount of I. As reported by Zhang et al. (2023) and Kiferle et al. (2021), it seems that I antioxidant activity involves iodinated proteins belonging to class III POD. Moreover, it was showed that I application regulates the expression of some genes comprised in plant defence response (Kiferle et al., 2021). In our study non-biofortified plants treated with biostimulants showed an increased antioxidant activity compared to the control, whereas biofortified plants treated with biostimulants revealed a reduction of the antioxidant activity of peel compared to the control. Consequently, since the antioxidant activity of peel was reduced by biostimulants application in biofortified plants, we may assume that biostimulants reduced the oxidative stress caused by I application.

Results on fruit I concentration agree with those obtained by Consentino et al. (2022) on eggplant. Remarkably, it was recorded an increase of I concentration in fruits when biostimulants were applied in combination with I. Indeed, control plants revealed always lower values than biostimulated ones. Consequently, we may assume that biostimulants act as a stress alleviator for eggplant, permitting a greater

assimilation and accumulation of I. It is of particular interest to underline that the combination of both biostimulants synergistically interacted in increasing I accumulation and tolerance in eggplant.

Findings on chlorophyll a, b and a+b are corroborated by other studies demonstrating a reduction of chlorophyll synthesis (Kiferle et al., 2021) and photosynthetic rate (Blasco et al., 2011) in I-treated plants. However, although it is well recognized that I is a chlorophyll component, the specific physiological mechanisms which interfere with photosynthesis remain unknown (Zhang et al., 2023). The experiment revealed that biostimulants significantly increased chlorophyll a, b and a+b concentration in plant tissues. As stated by Nardi et al. (2009), the positive effect of PH on chlorophylls can be attributed to its auxin-like ability and to the increase of N assimilation. On the other hand, AM effect on chlorophyll biosynthesis was also documented by Cartmill et al. (2007) and Kapoor and Bhatnagar (2007), who found a concentration increase of chlorophyll in AM-inoculated plants of Rosa multiflora and celery, respectively. Moreover, PH and AM worked synergistically in increasing chlorophyll concentration. Chlorophyll concentrations were also affected by the interaction between I and biostimulants. In this respect, biostimulants significantly enhanced chlorophyll traits, regardless of the I dose. Thus, the study revealed how PH and AM, especially when used in combination, can be helpful in reducing the deleterious impact of I on chlorophyll concentration and, consequently, on photosynthesis. Moreover, iodine application enhanced chlorophyll a/b ratio. Variations in chlorophyll a/b ratios can occur in reply to several stresses, such as nutrient restraint, light availability and osmotic balance alteration (Mulero et al., 2022). As previously reported by Ashraf et al. (2013), abiotic stresses - such as that caused by iodine - decrease chlorophyll a and b, while their ratio tend to increase; indeed, the preservation of more chlorophyll a than b is crucial for plant survival. Consequently, we can suppose that the increase of chlorophyll a/b ratio was a plant defence mechanism against iodine stress. The effect of biostimulants on chlorophyll a/b ratio could be ascribed to their influence on plant abiotic stress tolerance (Rouphael and Colla, 2020). Results on stomatal conductance can be due to a plant defence mechanism against I; indeed, the stomatal closure is often recorded in stressed plants (Li et al., 2022). Furthermore, findings are in accordance with those of Consentino et al. (2023a) and Augé et al. (2015) who revealed that PH and AM applications significantly increase plant stomatal conductance compared to non-treated plants. Attractively, the combination of PH and AM gave the highest results, suggesting a synergetic effect of these biostimulants on stomatal opening. Data also underlined that plants cultivated in year 2 had higher stomatal conductance values than those grown in year 1. This increase could be the effect of the higher temperatures recorded in year 2 during the first harvests. At this regard, in accordance with our study, Urban et al. (2017), who conducted a research on the effect of temperature on stomatal conductance, found a linear increase of the stomatal conductance as the temperature increases. It is attractive the impact of biostimulants on I negative effects; while there is a reduction in stomatal conductance as the dose of I increased, biostimulants emphasized stomatal conductance independently of the I supply, especially when plants were exposed to both biostimulants (PH+AM). These data provided insight regarding the buffer effect of the biostimulants against I toxicity.

In our research, proline concentration in plant tissues increased as I concentration in the nutrient solution increased. Consequently, we may speculate that the boost of proline biosynthesis is a plant defence mechanism against the oxidative stress caused by I. Our study also underlines that both biostimulants significantly enhanced proline concentration in plants. The effect of PH on proline can be explained by its composition since it contains proline and other amino acids like glutamic acid whose sodium salt (glutamate) is a precursor of proline (Alfosea-Simón et al., 2021). The effect of AM on proline accumulation has been largely documented (Chun et al., 2018) and it is attributed to the fungi symbiosis which trigger plant defence mechanisms and nutrient uptake enhancement. Remarkably, the highest proline

concentration was recorded in plants treated with both biostimulants, suggesting that the combined use of PH and AM can be a valuable strategy to increase proline concentration in plants and, consequently, plant resistance to abiotic stresses. Furthermore, data showed that I and biostimulants significantly interacted in altering proline concentration. The results indicated that biostimulants boosted proline concentration at any I dosages with a peak in plants treated with 600 mg L^{-1} of I and with PH+AM. These findings partly explain the valuable role of biostimulants in stress mitigation. Truly, since proline is biosynthesized to overcome abiotic stress, it can be hypothesized that the reduction in stress indicators - noted for other parameters - may be due, partly, to the augmented accumulation of proline in plant tissues.

Data underlined a significant H$_2$O$_2$ upsurge with the increase of I dosage. The recorded H$_2$O$_2$ increase might be linked to the oxidative stress caused by I in plants (Zhang et al., 2023). Data also revealed that biostimulants reduced H$_2$O$_2$ concentration in plants, with the most effective biostimulant being AM. This effect was also recorded by Trevisan et al. (2019), who found a H$_2$O$_2$ reduction in plants treated with *Fabaceae* hydrolysate. The mechanism behind this reduction could be the enhancement of plant detoxification mechanisms (superoxide dismutase), which prevents oxidative stress and presumably reduces the presence of H$_2$O$_2$ (Wang et al., 2018). Data on AM effect on H$_2$O$_2$ are corroborated by Hajiboland et al. (2010), who revealed that the inoculation significantly reduced H$_2$O$_2$ content in tomato. The decreasing effect recorded can be linked to the positive effect that AM had on reducing plant oxidative stress via the stimulation of plant defence mechanism (Li et al., 2020; Zou et al., 2021). Moreover, data underlined that the mutual application of PH and AM was the most effective in reducing H$_2$O$_2$ concentration in eggplant plant. The interaction between I and biostimulants was also recorded and provided important information on the plant anti-stress effect of biostimulants when treated with I.

Outcomes on malondialdehyde (MDA) are related to the oxidative stress caused by I applications. However, we recorded a significant decrease of MDA in plants treated with biostimulants compared to control, indicating the ability of biostimulants to reduce the oxidative plant stress (Molina et al., 2020; Jajoo and Mathur, 2021; Wen et al., 2020). Our results also revealed that I and biostimulant significantly interacted in modulating MDA concentrations. Data showed that PH is the most effective biostimulant for decreasing MDA concentration when plants are treated with 300 or 600 mg L^{-1} of I.

5. Conclusions

The current study evaluated the impact of two biostimulants, a plant protein hydrolysate and an arbuscular mycorrhizal fungus (used separately or in combination), to reduce the harmful impact of I on eggplant. Mainly, the results showed that the two biostimulants were effective in promoting eggplant performance and improving plant I tolerance, especially when jointly supplied. Overall, data highlighted that 300 mg L^{-1} of I combined with a mutual application of PH and AM can be a suitable protocol for eggplant I biofortification without jeopardizing fruit yield and quality traits. In the light of our results, further researches, such as omics studies, are required to investigate the mechanisms involved in the reduction of trace element stress in vegetables.

CRediT authorship contribution statement

Beppe Benedetto Consentino: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lorena Vultaggio:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Enrica Allevato:** Investigation, Formal analysis, Data curation. **Leo Sabatino:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Georgia Ntatsi: Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation. **Michele Ciriello:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Youssef Rouphael:** Writing – review & editing, Writing – original draft. **Giuseppe Di Miceli:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113437.

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