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Research Article

Effect of root imbibition with selenium and iodine on antioxidant compounds in tomato (*Solanum lycopersicum* L.) crop

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Abstract

The use of trace elements such as iodine and selenium in agriculture is gaining great importance due to the benefits in plants before different types of biotic or abiotic stress. This research aimed to evaluate the seedling root priming with Na_2SeO_3 (0, 0,5, 1, 2, 3 mg L^{-1}) and KIO_3 (0, 100, 150, 200, 250 mg L^{-1}) on the antioxidant compounds of tomato (*Solanum lycopersicum* L.) fruits and leaves. The crop was established under greenhouse conditions in 10-L polyethylene containers containing peat moss and perlite 1:1 (v/v), in a randomized complete block experimental design with a 5^2 factorial arrangement. In the fruits, the Na_2SeO_3 influenced the GHS, flavonoids, lycopene and β-carotene contents, while the KIO_3 influenced the GHS, vitamin C and lycopene contents. The KIO_3-Na_2SeO_3 interactions affected the GSH, phenols, flavonoids, lycopene and β-carotene contents in fruits. In the leaves the GHS content increased with the Na_2SeO_3, while the GSH, flavonoids, and chlorophyll contents increased with the KIO_3 factor and KIO_3-Na_2SeO_3 interactions. The evaluated enzymes in fruits and leaves decreased with the both the KIO_3 and Na_2SeO_3 concentrations. The Na_2SeO_3 influenced the hydrophilic compounds by ABTS and DPPH, while the KIO_3 influenced the hydrophilic compounds by ABTS. In the leaves, the KIO_3 influenced the lipophilic compounds by ABTS. The KIO_3-Na_2SeO_3 interactions influenced the hydrophilic compounds by ABTS in both the fruits and leaves. Seedling root imbibition in KIO_3 and Na_2SeO_3 is a method that implemented in the tomato crop presents interesting aspects in the increase of the antioxidant capacity and the non-enzymatic compounds, such

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as vitamin C, phenols, flavonoids and GSH contents. However, this method presented an inhibition in the antioxidant enzymes.

Keywords: antioxidant; KIO_3; Na_2SeO_3; ROS; secondary metabolites

Introduction

The use of trace elements such as iodine (I) and selenium (Se) in agriculture is a practice that is gaining great importance and relevance worldwide, because I and Se can promote growth and the potential for tolerance to plant stress when applied in low concentrations (Hasanuzzaman *et al*., 2010). Selenium (Se) is classified as an inorganic plant biostimulant, it has shown an improvement in the absorption of nutrients, increases the tolerance of plants to stress and improves the quality of crop yields (Dima *et al*., 2020; Hasanuzzaman *et al*., 2020; de Mello Prado, 2021). I and Se in low concentrations can function as signalers to improve the plant's defense system, which is reflected in the increase in the content of secondary metabolites; however, in high concentrations it can cause oxidative damage to tissues (Mittler, 2017; Abedi *et al*., 2021).

Plants have the ability to absorb chemical elements from the soil and from the nutrient solution, whether these are nutrients or non-nutrients, as well as beneficial or toxic (Kathpalia and Bhatla, 2018). Se is absorbed by plant roots in the rhizosphere solution in its organic form as selenocysteine (SeCys) and selenomethionine (SeMet), and its inorganic form as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), but selenides or elemental Se cannot be absorbed by plant roots (White, 2018).

Plants are capable of absorbing selenate and selenite ions in the root; however, none of the ions are absorbed through a specific Se transporter. Selenate is absorbed through the H^+/sulfate importer. Sulfate transporters SULTR1;1 and SULTR1;2 are high-affinity transporters that absorb sulfate in the root, and have been shown to be capable of transporting selenate, while the selenite is taken up by inorganic phosphate (Pi) transporters and aquaporins (Schiavon and Pilon-Smits, 2017; White, 2018; Trippe and Pilon-Smits, 2021).

Iodine can be absorbed from the soil through the plant roots as organic iodine ions, iodate (IO_3) and iodide (I), and from the atmosphere in gaseous form by the plant leaves as molecular iodine (I_2) and methyl iodide (CH_3I) (Medrano-Macías *et al*., 2016).

Iodine and selenium play important roles with benefit in crop plants particularly under stress conditions, presenting positive effects in reducing the H_2O_2 and O_2^- contents (Zhu *et al*., 2017), that is, have an effect on the activation of the defense system to control the production and accumulation of reactive oxygen species (ROS). In this context, the plant cell system increases the levels of non-enzymatic antioxidant metabolites, including glutathione, ascorbate, tocopherol, phenolic compounds, anthocyanins (Halka *et al*., 2019; Huang *et al*., 2019), and a wide network of enzymatic antioxidants, such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX) and glutathione reductases (GR), among others (Mittler *et al*., 2004; Revelou *et al*., 2022).

Tomato (*Solanum lycopersicum* L.) is a horticultural crop of worldwide importance due to its wide consumption as a processed byproduct and fresh presentation. This research aimed to evaluate the effect of seed priming based on I and Se on the antioxidant compounds of tomato fruits and leaves.

Materials and Methods

Crop establishment

Tomato crop was established in a tunnel-type greenhouse with plastic cover and natural ventilation in the Horticulture Department at the Universidad Autónoma Agraria Antonio Narro, in Saltillo, Mexico (25°

21' NL, 101° 01' WL, altitude 1743 m). The average conditions in the greenhouse in the crop cycle were: temperature 21 °C, relative humidity 51%, solar radiation 735 W m^{-2}, photosynthetically active radiation 568 μmol m^{-2} s^{-1}.

Preparation of treatments

Sodium selenite (Na_2SeO_3) (99%, Sigma Aldrich, St. Louis, MO, USA) and Potassium iodate (KIO_3) (99%, Sigma Aldrich, St. Louis, MO, USA) and were used. A sodium selenite stock solution (1000 ppm) was prepared. A mass of 21,89 mg of Na_2SeO_3 was gauged to 10 mL with distilled water. Dilutions of 0,5, 1, 2 and 3 mL of the stock solution were gauged to 1 L with distilled water to obtain the treatments of 0,5, 1, 2, and 3 mg L^{-1}, respectively. Also, a potassium iodate stock solution (1000 ppm) was prepared. A mass of 1,68 g of KIO_3 was gauged to 1 L with distilled water, and the dilutions of 100, 150, 200 and 250 mL of the stock solution were gauged to 1 L with distilled water to obtain the treatments of 100, 150, 200, and 250 mg L^{-1}, respectively. Control treatments consisted of imbibition in distilled water (Table 1).

Table 1. Imbibition treatments of tomato root with Se and I

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})
0	0
0.5	100
1	150
2	200
3	250

25 treatments (5^2 factorial), n = 4 replications, 100 experimental units.

Sowing and root priming

Saladette-type CID F1 (Harris Moran®, Davis, CA, USA) tomato seeds were sown in polystyrene trays with a substrate mixture of peat moss and perlite 1:1 (v/v). The seedling roots of tomato were primed in KIO_3 and Na_2SeO_3 osmotic solutions at the 35 days after sowing, and the seedling roots of the control treatment were primed in distilled water (Table 1), by a 24-h imbibition time.

Planting and crop management

Tomato seedlings were planted at the 36 days after sowing in 10-L plastic containers with a substrate mixture of peat moss and perlite 1:1 (v/v). Tomato plants were arranged into the greenhouse in a randomized complete block design with a factorial arrangement of two factors (KIO_3 and Na_2SeO_3) and five levels (concentrations in mg L^{-1}) (Table 1). Fertilization consisted of Steiner-type nutrient solution (Steiner, 1961) diluted in drip irrigation. The same substrate and fertigation conditions were used in the control, and KIO_3 and Na_2SeO_3 treatments to avoid another variation factor affecting the treatments performance.

Sampling

Samples of leaves and ripe fruits of tomato plants were obtained 120 days after planting. Leaf samples were collected from the leaf tissue of fully extended young leaves from 12 plants with four replications. Samples for biochemical analysis were collected from five ripe fruits per treatment, with a uniform color and size corresponding to stage six of ripening (USDA, 2017). Samples were stored at -80 °C, lyophilized in a 2,5 L FreeZone Benchtop Free Dry System freeze-dryer (LABCONCO, Kansas, MO, USA) and ground to a fine powder.

Non-enzymatic compounds

Vitamin C

The vitamin C content was determined by the 2,6 dichlorophenolindophenol titration method (Padayatty *et al*., 2001). The results were computed (Equation 1) and expressed in mg per 100 g of fresh tissue (mg 100 g^{-1} FW).

$$\text{Vitamin C} = \frac{\text{mL of 2,6 dichlorophenolindophenol} \times 0{,}088 \times \text{total volume} \times 100}{\text{aliquot volume} \times \text{sample weight}} \quad (1)$$

Phenols

Phenolic compounds were determined using the Folin–Ciocalteu method from the extraction with water:acetone according to Yu and Dahlgren (2000). The results were expressed in mg of gallic acid equivalents per gram of dry tissue (mg GAE g^{-1} DW).

Flavonoids

Flavonoids were determined by the Dowd method, adapted by Arvouet-Grand *et al*. (1994). The results were expressed in mg of quercetin equivalents per 100 g of dry tissue (mg QE 100 g^{-1} DW).

Chlorophyll

Chlorophyll content was quantified using the method proposed by Munira *et al*. (2015), by reading the absorbances at 663 and 645 nm wavelengths, and the results were computed (Equations 2, 3 and 4) and expressed in µg per gram of fresh tissue (µg g^{-1} FW).

$$Chlorophyll\text{–}a = 3.64 \times A_{645} + 25.38 \times A_{663} \quad 2)$$

$$Chlorophyll\text{–}b = 30.38 \times A_{645} - 6.58 \times A_{663} \quad 3)$$

$$Total\ chlorophyll = Chlorophyll\text{–}a + Chlorophyll\text{–}b \quad 4)$$

Lycopene and β-carotene

Lycopene and β-carotene contents were determined according to Nagata and Yamashita (1992), by reading the absorbances at 453, 505, 645, and 663 nm wavelengths. The results were computed (Equations 5 and 6) and expressed in mg per 100 g of dry tissue (mg 100 g^{-1} DW).

$$Lycopene = -0.0806 \times A_{453} + 0.372 \times A_{505} + 0.204 \times A_{645} - 0.0458 \times A_{663} \quad 5)$$

$$\beta\text{–}carotene = 0.452 \times A_{453} - 0.304 \times A_{505} - 1.22 \times A_{645} + 0.216 \times A_{663} \quad 6)$$

Extraction

Samples of leaves and rape fruits of tomatoes were freeze-dried and macerated by using a mortar and pestle; 200 mg of dry tissue and 20 mg of polyvinyl pyrrolidone were added in a 2-mL centrifuge tube; 1,5 mL of phosphate buffer (0,1 M, pH 7-7,2) was added; and the mixture was subjected to sonication for 5 min, and then centrifuged in a Prism C2500 refrigerated microcentrifuge (Labnet International Inc., Edison, NJ, USA) at 12,500 rpm for 10 min at 4 °C. The supernatant was collected and filtered with a 0,45-mm-diameter nylon membrane. Finally, the supernatant was diluted (1:20) with phosphate buffer (0,1 M, pH 7-7,2). This dilution was used to analyze the absorbances of reduced glutathione (GSH), glutathione peroxidase (GPX), phenylalanine ammonium lyase (PAL), catalase (CAT), and ascorbate peroxidase (APX) in a GENESYS 10S UV-Vis Spectrum (Thermo Fisher Scientific, Inc., Waltham, MA, USA), as well as the antioxidant capacity of

ABTS and DPPH radicals in a BK-EL10C Elisa microplate reader (BIOBASE, Jinan, Shandong, China) at the corresponding wavelengths.

Reduced Glutathione (GSH)

GSH quantification was performed by the spectrophotometric technique (Xue *et al*., 2001). The results were expressed in units per gram of total protein (U g^{-1} TP), where U is equal to mM of GSH equivalents per mL per minute of dry tissue (mM GSHE mL^{-1} min^{-1} DW).

Enzymatic Activity

Glutathione Peroxidase (GPX) (QE 1.11.1.9)

GPX was determined using the Flohé and Günzler (1984) method, adapted by Xue *et al*. (2001). The results were expressed in units per gram of total protein (U g^{-1} TP), where U is equal to mM of GSH equivalents per mL per minute of dry tissue (mM GSHE mL^{-1} min^{-1} DW).

Phenylalanine Ammonium Lyase (PAL) (QE 4.3.1.5)

PAL was determined according to Sykłowska-Baranek *et al*. (2012). The results were expressed in units per 100 g of total protein (U 100 g^{-1} TP), where U is equal to µmol of trans-cinnamic acid equivalents per mL per minute of dry tissue (µmol TCAE mL^{-1} min^{-1} DW).

Catalase (CAT) (QE 1.11.1.6)

CAT was determined by the spectrophotometric method Dhindsa *et al*. (1981). The results were expressed in units per gram of total protein (U g^{-1} TP), where U is equal to mM of H_2O_2 equivalents spent per mL per minute of dry tissue (mM H_2O_2E mL^{-1} min^{-1} DW).

Ascorbate Peroxidase (APX) (EC 1.11.1.11)

APX quantification was performed according to the Nakano and Asada, (1987) method. The results were expressed in units per gram of total protein (U g^{-1} TP), where U is equal to µmol of ascorbate oxidized equivalents per mL per minute of dry tissue (µmol AOE mL^{-1} min^{-1} DW).

Antioxidant capacity

Hydrophilic and Lipophilic Compounds by ABTS

Antioxidant activity by the ABTS radical (2,2′-azino-bis-3-ethylbenzothiazolin-6-sulfonic acid) was determined by the spectrophotometric method (Re *et al*., 1999). Both the antioxidant capacity results of hydrophilic and lipophilic compounds by ABTS were expressed in µmol of Trolox equivalents per gram of dry tissue (µmol TE g^{-1} DW).

Hydrophilic Compounds by DPPH

Antioxidant capacity by DPPH radical (2,2-Diphenyl-1-picrylhydrazyl) was performed according to Brand–Williams *et al*. (1995). The antioxidant capacity results of hydrophilic compounds by DPPH were expressed in µmol of Trolox equivalents per gram of dry tissue (µmol TE g^{-1} DW).

Statistical analyses

The results were analysed by analysis of variance to determine the variables that presented a significant statistical difference ($p \leq 0{,}05$) so that the variables with significant effects were submitted to comparison means tests by Tukey ($p \leq 0{,}05$) using the statistical software InfoStat® 2020e.

Results and Discussion

Non-enzymatic compounds in tomato fruits by Na_2SeO_3

Regarding the sodium selenite factor in the tomato fruits, the GSH content significantly increased 5,6 times in the dose of 3 mg L^{-1}, while the vitamin C content significantly decreased in the doses of 0,5, 1 and 2 mg L^{-1}, in relation to the control treatments. On the other hand, the total phenols content significantly increased by 4 and 9% in the doses of 0,5 and 3 mg L^{-1} in relation to the control treatment, similar results were reported by Andrejiová *et al*. (2016) and Abedi *et al*. (2021), who indicated that the use of selenium favors the increase of polyphenols in the tomato crop. The flavonoid, lycopene and ß-carotene contents significantly increased by 22, 52 and 127%, respectively, in the dose of 0,5 mg L^{-1} in relation to the control treatments (Figure 1), similar results were reported by Rady *et al*. (2020), who indicated that the use of selenium favors the increase in the lycopene content in tomato fruits, while Sabatino *et al*. (2021) indicated that the use of selenium presents important aspects in the nutraceutical quality in tomato fruits, presenting increases in the carotenoid, polyphenol, vitamin C and lycopene contents.

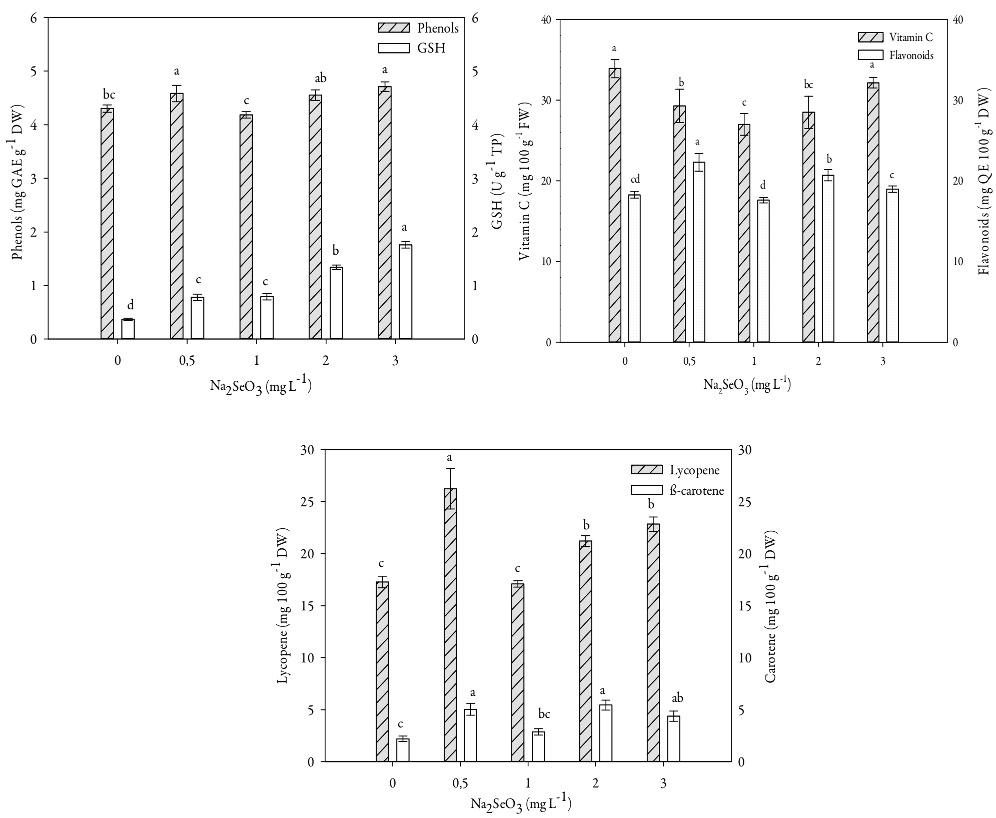

Figure 1. Effect of root imbibition by Na_2SeO_3 in the non-enzymatic antioxidant compounds in tomato fruits

Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Non-enzymatic compounds in tomato fruits by KIO_3

The root imbibition with potassium iodate significantly influenced the contents of non-enzymatic compounds in the tomato fruits. The GSH and vitamin C contents increased by 37 and 11,7%, respectively, in the dose of 200 mg L^{-1}, in relation to the corresponding control treatments. Li *et al*. (2017a) reported that the use of iodine concentrations favors the increase in vitamin C content in chili crop, which tends to decrease at high iodine concentrations. This stimulation effect of iodine at low concentrations to increase the vitamin C content also was observed in strawberry crop by Li *et al*. (2017b) with applications either as iodide or iodate in the nutrient solution in concentrations lower than 1 mg L^{-1}, while higher iodine concentrations influenced the decrease the ascorbic acid content in relation to the control treatment. The phenols, flavonoids and lycopene contents increased in the dose of 200 mg L^{-1}, while the β-carotene content was not significantly affected in relation to the control treatments (Figure 2). Opposite results were reported by Smoleń *et al*. (2015), where the use of KI and KIO_3 did not significantly influence the carotenoids, flavonoids and phenols contents in relation to the control treatments.

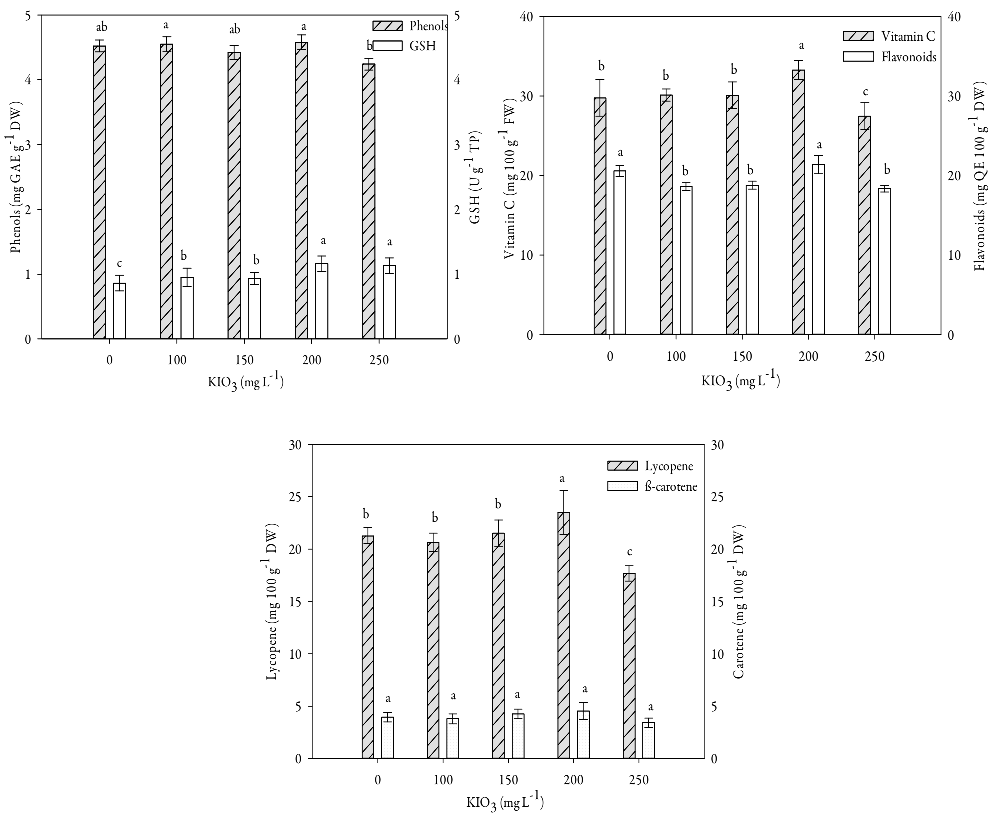

Figure 2. Effect of root imbibition by KIO_3 in the non-enzymatic antioxidant compounds in tomato fruits
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0{,}05$). n = 4.

Non-enzymatic compounds in tomato fruits by Na_2SeO_3 and KIO_3 interactions

The GSH content in the tomato fruits significantly increased 9 times in the 3-100 mg L^{-1} interaction, in relation to the control treatment (0-0 mg L^{-1} interaction). The vitamin C content tended to significant decrease by 56,4% of in the 2-250 mg L^{-1} in relation to the 2-0 mg L^{-1} interaction implies that KIO_3 have a negative effect in the ascorbic acid content when it is combined with Na_2SeO_3 at 2 mg L^{-1}. The 0,5-200 mg L^{-1} interaction significantly influenced the increase of phenols, flavonoids, lycopene and β-carotenes contents in 1,23, 1,66, 2,13 and 2,76 times, respectively, in relation to the control treatments (Table 2), for which this interaction presents important aspects in nutraceutical quality and postharvest quality, because the flavonoid contributes to delay the ripening of tomato fruits (Zhang *et al*., 2015).

Table 2. Effect of root imbibition by Na_2SeO_3 and KIO_3 interactions in the non-enzymatic antioxidant compounds in tomato fruits

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})	GSH (U g^{-1} TP)	Vitamin C (mg 100 g^{-1} FW)	Phenols (mg AG g^{-1} DW)	Flavonoids (mg QE 100 g^{-1} DW)	Licopene (mg 100 g^{-1} DW)	β-carotene (mg 100 g^{-1} DW)
0	0	0.2k	37.0abc	4.3bcde	18,6defghi	17.6ghijk	2.9b
0	100	0.3jk	28.4def	4.4abcde	16.4hi	17.6ghijk	1.8b
0	150	0.3jk	39.1a	3.8de	18.1defghi	16.1jk	2.0b
0	200	0.4ijk	34.2abcd	4.3bcde	20.6cdef	19.7efghijk	1.7b
0	250	0.5ij	30.7cdef	4.4abcde	17.4fghi	15.0k	2.4b
0,5	0	0.5i	15.8h	4.4abcde	20.0cdefg	25.5bc	4.1ab
0,5	100	0.6hi	32.3bcde	4.4abcde	19.6cdefgh	23.6cdef	4.2ab
0,5	150	0.8gh	31.1cdef	4.9abc	22.5bc	28.9b	6.5ab
0,5	200	1.2de	38.6ab	5.3a	30.9a	37.4a	8.0a
0,5	250	0.6ghi	28.4def	3.7e	18.1defghi	15.4jk	2.1b
1	0	0.5ij	24.6fg	4.1cde	17.9defghi	17.9ghijk	1.7b
1	100	0.5ij	26.7efg	3.9de	16.0i	17.3hijk	2.6b
1	150	0.8fg	21.1gh	4.2cde	17.9defghi	17.0ijk	3.5ab
1	200	0.8fg	36.0abc	4.4abcde	19.0defghi	15.6jk	3.0b
1	250	1.1de	26.4efg	4.1cde	16.9ghi	17.5ghijk	3.3ab
2	0	1.5b	39.0a	5.1ab	25.2b	22.5cdefg	6.4ab
2	100	1.3bcd	28.9def	4.6abcde	20.5cdef	20.1defghij	5.9ab
2	150	1.1ef	30.8cdef	4.4bcde	17.5fghi	22.2cdefgh	4.1ab
2	200	1.2cde	26.5efg	4.4bcde	18.7defghi	19.3efghijk	5.7ab
2	250	1.4bc	17.0h	4.2cde	21.3cd	21.6cdefghi	4.9ab
3	0	1.3bcd	32.3bcde	4.5abcde	21.0cde	22.5cdefg	4.3ab
3	100	1.8a	34.1abcd	5.3a	20.5cdef	24.4bcde	4.1ab
3	150	1.5b	28.3def	4.6 abcd	17.7efghi	23.2cdef	4.9ab
3	200	2.0a	31.1cdef	4.3bcde	17.5fghi	25.2bcd	4.2ab
3	250	1.9a	34.7abcd	4.6abcd	17.9defghi	18.6fghijk	4.1ab

Different letters within the columns indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$; n = 4).

Enzymatic compounds in tomato leaves by Na_2SeO_3

The seedling root imbibition with sodium selenite significantly influenced the increase of the GSH content in tomato leaves, to a maximum increase of four times in the dose of 3 mg L^{-1} in relation to the control treatment. Rady *et al.* (2020) found that the use of selenium influences the increase of GSH content in tomato seedlings, while Dall'Acqua *et al.* (2019) indicated that the use of exogenous selenium may influence the GSH content according to the crop specie and the application dose. The phenol content significantly increased 3,5 times in the dose of 3 mg L^{-1} in relation to the control treatment. The flavonoid content was not significantly modified by Na_2SeO_3 (Figure 3), Zhang *et al.* (2023) also reported not significant results in the flavonoid content with Na_2SeO_3 applied by foliar spraying and through irrigation. The chlorophyll contents significantly decreased in the dose of 0,5 mg L^{-1} in relation to the control treatments. Huang *et al.* (2018), Khalofah *et al.* (2021), and El-Badri *et al.* (2022), also reported the decrease in chlorophyll and β-carotene contents as the Na_2SeO_3 concentration was higher; however, Alsamadany *et al.* (2023) indicated that the use of selenium favored the increase in the chlorophyll content, while the β-carotene content was not significantly modified, in relation to the control treatments. Cunha *et al.* (2022) and Ishtiaq *et al.* (2023) indicated that the use of selenium influences the increase of chlorophyll, β-carotene and phenolic compounds with concentrations from 7,5 to 15 μg kg^{-1}, whereas the selenium concentration was higher the content of the variables began to decrease. The Se at low concentrations decreases the leaf senescence rate and the peroxidase activity, which can increase the nitrogen utilization efficiency and benefiting the crop production. However, in high concentrations the Se promotes oxidative stress, nutritional disturbance, damaging photosynthesis (da Cruz Ferreira *et al*., 2020), inducing symptoms of toxicity in the plant, reducing growth, causing foliar chlorosis and small and brittle roots (de Mello Prado, 2021).

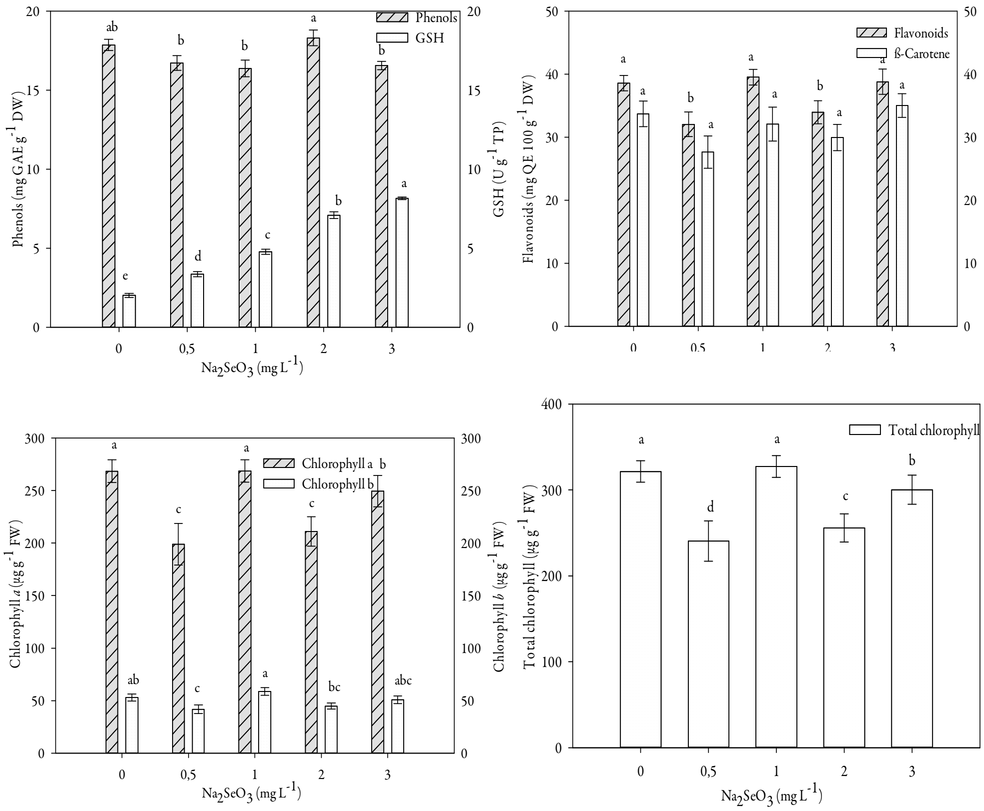

Figure 3. Effect of root imbibition by Na_2SeO_3 in the non-enzymatic antioxidant compounds in tomato leaves

Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Non-enzymatic compounds in tomato leaves by KIO_3

In the tomato leaves the potassium iodate significantly influenced an increase of 26% of the GSH content in the dose of 250 mg L^{-1}, in relation to the control treatment. The phenol content was not significantly modified by KIO_3, similar results are those reported by Puccinelli *et al*. (2021) who indicated that the use of KI in the lettuce crop did not a significant influence the phenol content. The flavonoid content significantly increased by 9,8% in the dose of 100 mg L^{-1} of KIO_3 in relation to the control treatment. The greater chlorophyll contents were presented in the dose of 100 mg L^{-1} of KIO_3 in relation to the control treatments, these results agree with those reported by Li *et al*. (2017a) who indicated that the use of iodine improves the chlorophyll content, however, as the iodine concentration increases, the chlorophyll content begins to decrease. Regarding the β-carotene content, there was no significant difference between the treatments (Figure 4). Krzepilko *et al*. (2023) indicated that the use of iodine influences the increase in the chlorophyll content, however, in the carotenoid content, the applications either as KI or KIO_3 do not influence the carotenoid content.

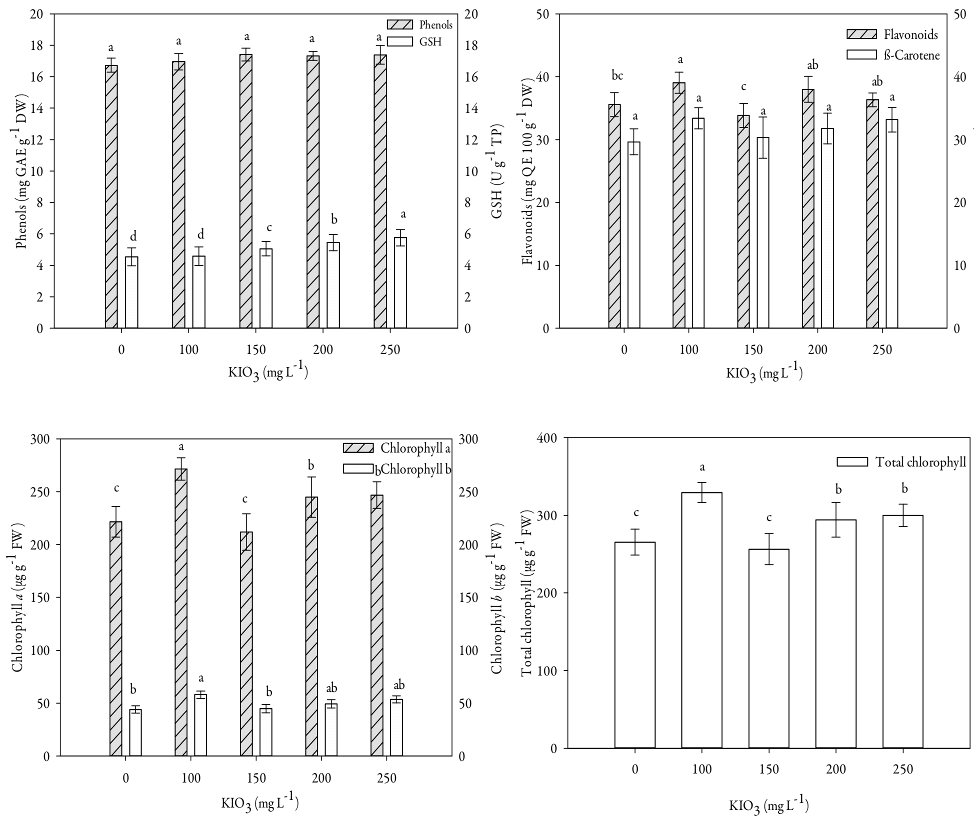

Figure 4. Effect of root imbibition by KIO_3 in the non-enzymatic antioxidant compounds in tomato leaves
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0{,}05$). n = 4.

Non-enzymatic compounds in tomato leaves by Na_2SeO_3 and KIO_3 interactions

The higher significant GSH content in the tomato leaves reached 7,25 times in the 2-250 mg L^{-1} (Na_2SeO_3 - KIO_3) interaction in relation to the control treatment (0-0 mg L^{-1} interaction), which evidences the importance of the glutathione role in the control of reactive oxygen species (ROS) that accumulate during biotic stress, and the GSH reduces the cell damage (Gullner *et al*., 2017; Zechmann, 2020). The phenol and β-carotene contents in the tomato leaves did not show significant effects. Regarding the flavonoid content, the tomato leaves reached an increase of 41,2% in the 0,5-100 mg L^{-1} interaction, and 51,8% in the 3-200 mg L^{-1} interaction, in relation to the control treatment, however the 10,5% surplus implies an increase in the cost by 6(Na_2SeO_3) + 2(KIO_3). Golubkina *et al*. (2018) reported that the use of iodine and selenium together influence favorable effects by increasing the content of flavonoids, while Smoleń *et al*. (2019) indicated that the application of iodine and selenium together influence the increase of plant metabolism, which is reflected in the increase of antioxidant compounds in the face of the stress by which the plants are found. The chlorophyll-*a*, chlorophyll-*b* and total chlorophyll contents significantly increased by 34, 80,6 and 40,9%, respectively, in the 1-100 mg L^{-1} interaction, compared to the control treatments (Table 3).

Table 3. Effect of root imbibition by Na_2SeO_3 and KIO_3 interactions in the non-enzymatic antioxidant compounds in tomato leaves

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})	GSH (U g^{-1} TP)	Phenols (mg AG g^{-1} DW)	Flavonoids (mg QE 100 g^{-1} DW)	Chl-*a* (µg g^{-1})	Chl-*b* (µg g^{-1})	Total Chl. (µg g^{-1})	β carotene (mg 100 g^{-1} DW)
0	0	1.2i	17.3abc	31.3efghi	231.3efg	40.2b-e	271.5fg	29.5a
0	100	1.4hi	17.3abc	42.8ab	309.3ab	62.9ab	372.2a	35.2a
0	150	2.1gh	17.3abc	43.2ab	298.3abc	60.5abc	358.9abc	38.6a
0	200	2.2gh	18.1abc	41.8abc	300.8abc	57.4a-d	358.3a-d	33.7a
0	250	2.9g	19.1ab	33.5cdefgh	201.6fgh	44.4a-e	246.0gh	31.1a
0,5	0	2.8g	16.4abc	29.7ghi	162.4h-k	36.3b-e	198.7ij	24.4a
0,5	100	2.2gh	15.8abc	44.2ab	284.8a-d	59.5abc	344.3a-d	36.7a
0,5	150	3.9f	19.0ab	24.3i	118.9kl	26.4de	145.3k	19.5a
0,5	200	3.8f	17.4abc	23.7i	111.5l	24.4e	135.9k	19.8a
0,5	250	3.8f	14.8bc	38.0bcdefg	315.9a	62.8abc	378.8a	37.6a
1	0	4.0f	14.1c	45.8ab	298.9abc	63.2ab	362.2ab	31.8a
1	100	4.2f	15.4abc	42.3abc	309.9ab	72.6a	382.5a	31.8a
1	150	4.6ef	17.3abc	37.5bcdefg	268.6a-e	55.2a-e	323.9b-e	34.7a
1	200	5.4de	17.0abc	32.5defghi	184.8ghi	40.7b-e	225.5hi	30.0a
1	250	5.4de	17.9abc	39.3abcdef	280.2a-d	62.3abc	342.6a-d	31.7a
2	0	6.2cd	18.9ab	26.6hi	139.6i-l	31.7b-e	171.3jk	22.4a
2	100	6.7c	19.5a	27.6hi	189.8gh	39.6b-e	229.4hi	28.0a
2	150	6.6c	16.9abc	40.3abcd	240.6def	50.2a-e	290.8ef	34.3a
2	200	7.1bc	16.7abc	44.4ab	310.6ab	62.7abc	373.4a	36.6a
2	250	8.7a	19.2ab	30.6fghi	174.1hij	39.8b-e	214.0hi	28.1a
3	0	8.3a	16.7abc	44.3ab	275.0a-e	48.5a-e	323.5b-e	39.8a
3	100	8.1a	16.6abc	38.2b-g	262.7b-e	55.4a-e	318.2de	35.0a
3	150	7.8ab	16.3abc	23.7i	131.9jkl	31.2cde	163.1jk	24.3a
3	200	8.5a	17.2abc	47.5a	316.2a	61.3abc	377.5a	38.6a
3	250	7.9ab	15.8abc	40.1abcde	261.0cde	58.0a-d	319.0cde	37.2a

Different letters within the columns indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Enzymatic compounds of tomato fruits by Na_2SeO_3

The sodium selenite applied by imbibition to the seedling roots negatively influenced the enzymatic activity in the tomato fruits. The GPX, PAL, CAT y APX enzymatic activities significantly decreased by 73,1, 68,1, 85,3 and 73,9%, respectively, in the dose of 3 mg L^{-1} in relation of the control treatments (Figure 5), which indicates that Na_2SeO_3 applications by root imbibition influence toxicity problems by inhibiting the enzymatic activities, because the ROS concentrations exceed the cellular detoxification capacity, cause oxidative stress, increase the oxidation of molecules such as DNA, proteins, lipids and carbohydrates (Sali *et al*., 2018; Berni *et al*., 2019). An alternative for improving the enzymatic activity in tomato cultivation from the root priming is the use of Se nanoparticles (Ishtiaq *et al*., 2023).

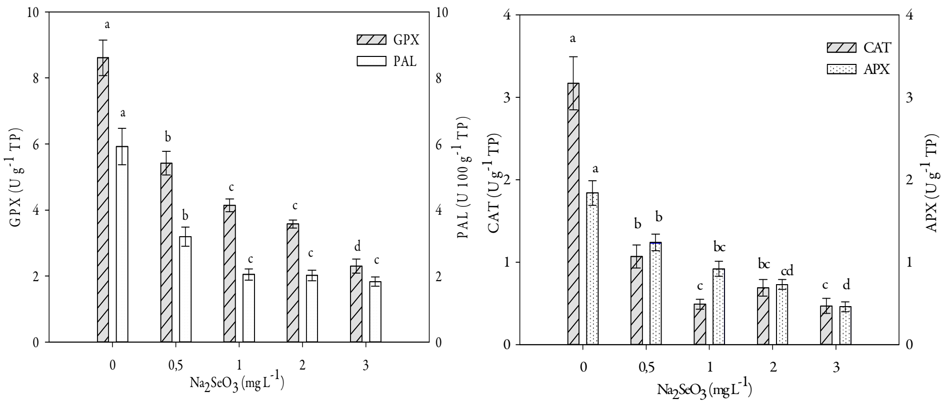

Figure 5. Effect of root imbibition by Na_2SeO_3 in the enzymatic compounds in tomato fruits
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0{,}05$). n = 4.

Enzymatic compounds of tomato fruits by KIO_3

The potassium iodate applied by imbibition to the seedling roots negatively influenced the enzymatic activity in the tomato fruits. The GPX, PAL, CAT y APX enzymatic activities significantly decreased by 23,8, 42,5, 53,1 and 33,3%, respectively, in the dose of 250 mg L^{-1} in relation of the control treatments (Figure 6). The CAT and APX enzymes are responsible for degrading H_2O_2 in water, such process was inhibited due to enzymatic activity was decreased as the KIO_3 dose increased.

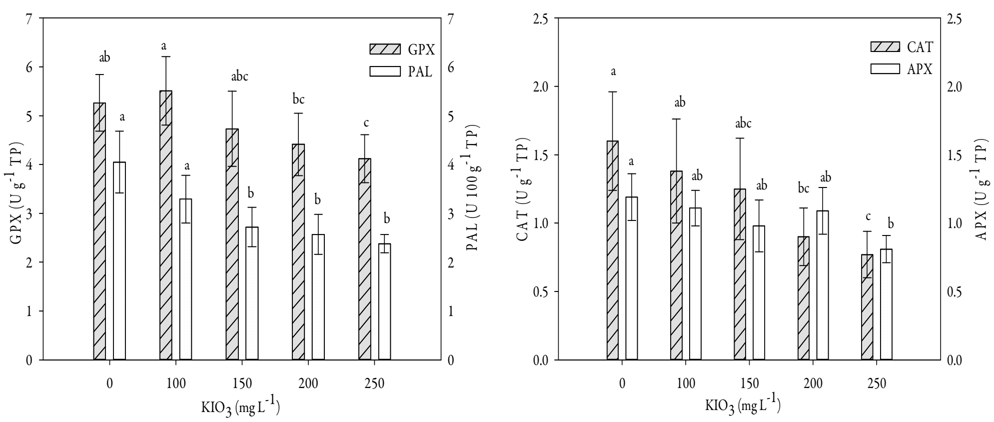

Figure 6. Effect of root imbibition by KIO_3 in the enzymatic compounds in tomato fruits
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0{,}05$). n = 4.

Enzymatic compounds of tomato fruits by Na_2SeO_3 and KIO_3 interactions

The enzymatic activity in the tomato fruits significantly decreased as the doses of treatments of Na_2SeO_3 and KIO_3 interaction increased (Table 4). The enzymes in the plant reduce the level of reactive oxygen species (ROS), where catalase degrades H_2O_2 into oxygen and water, while ascorbate peroxidase uses ascorbic acid as a donor to stimulate the H_2O_2 degradation, while reduced glutathione is responsible for the production of ascorbic acid (Zhu *et al*., 2017; Hussain *et al*., 2019).

Table 4. Effect of root imbibition by Na_2SeO_3 and KIO_3 interactions in the enzymatic compounds in tomato fruits

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})	PAL (U 100 g^{-1} TP)	GPX (U g^{-1} TP)	CAT (U g^{-1} TP)	APX (U g^{-1} TP)
0	0	8.8a	7.2abc	4.1a	1.9ab
0	100	7.0ab	10.0a	3.9a	1.8abc
0	150	5.5bc	10.0a	3.6ab	2.1a
0	200	4.9bcd	8.5ab	2.2bc	1.9ab
0	250	3.1cde	7.1abc	1.8cd	1.3abcde
0,5	0	4.0cde	7.1abc	1.6cd	1.7abcd
0,5	100	3.2cde	6.3bcd	1.1cd	1.0abcde
0,5	150	2.7cde	4.5cde	1.0cd	0.9bcde
0,5	200	3.5cde	4.7cde	0.8cd	1.3abcde
0,5	250	2.4de	4.3cde	0.7cd	1.0abcde
1	0	2.5de	4.7cde	0.4d	1.1abcde
1	100	2.0e	4.8cde	0.5d	1.2abcde
1	150	1.9e	3.9de	0.5d	0.8cde
1	200	1.1e	3.1de	0.5d	0.6de
1	250	2.5de	4.1cde	0.4d	0.7cde
2	0	2.1de	3.5de	1.1cd	0.6de
2	100	2.1de	3.8de	0.6d	0.8bcde
2	150	1.9e	3.3de	0.5d	0.6de
2	200	1.4e	3.9de	0.5d	0.9bcde
2	250	2.4de	3.3de	0.6cd	0.5e
3	0	2.6de	3.6de	0.7cd	0.5e
3	100	1.9e	2.5e	0.6cd	0.5e
3	150	1.4e	1.8e	0.3d	0.3e
3	200	1.7e	1.7e	0.3d	0.5de
3	250	1.3e	1.6e	0.2d	0.3e

Different letters within the columns indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Enzymatic compounds of tomato leaves by Na_2SeO_3

The sodium selenite applied by imbibition to the seedling roots negatively influenced the enzymatic activity in the tomato leaves. The GPX, PAL, CAT y APX enzymatic activities significantly decreased by 50, 75, 70 and 97,7%, respectively, in the dose of 3 mg L^{-1} in relation of the control treatments (Figure 7). Rady *et al*. (2020) indicated that the use of Na_2SeO_3 at 25 and 50 mM applied by foliar spraying influence the increase of enzymatic activity, while Cunha *et al*. (2022) reported an increase in the CAT and APX enzymatic activities in the lowest concentration (7,5 µg kg^{-1}), and as the concentration increased the enzyme activities decreased.

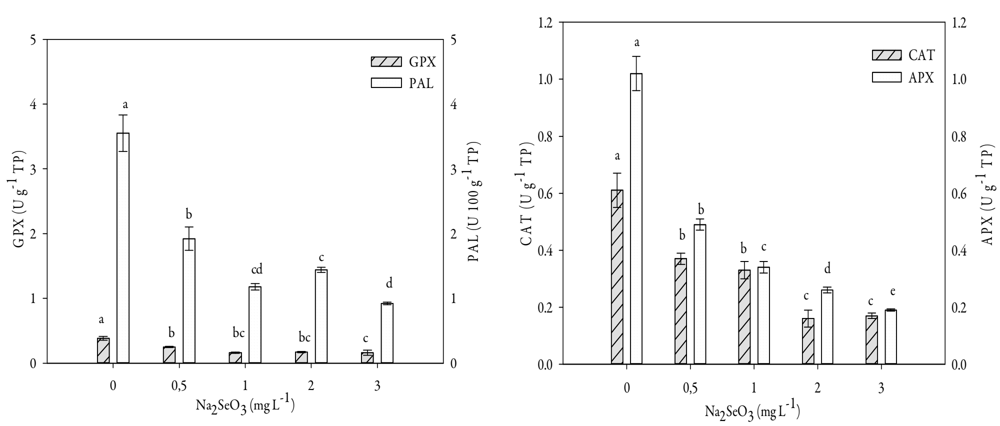

Figure 7. Effect of root imbibition by Na_2SeO_3 in the enzymatic compounds in tomato leaves
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Enzymatic compounds of tomato leaves by KIO_3

The potassium iodate applied by imbibition to the seedling roots negatively influenced the enzymatic activity in the tomato leaves. The GPX, PAL, CAT y APX enzymatic activities significantly decreased by 2,3, 34,1, 11,5 and 31,7%, respectively, in the dose of 250 mg L^{-1} in relation of the control treatments (Figure 8), which reflects that in higher concentrations the iodine presents an oxidative stress, due to the CAT and APX are enzymatic antioxidants can catalyze the decomposition of H_2O_2 into H_2O and O_2, which protect the cells from excess H_2O_2. Li *et al*. (2017a) reported that as higher the iodine concentration in seedlings, the enzymatic activity is lower in relation of the control treatment.

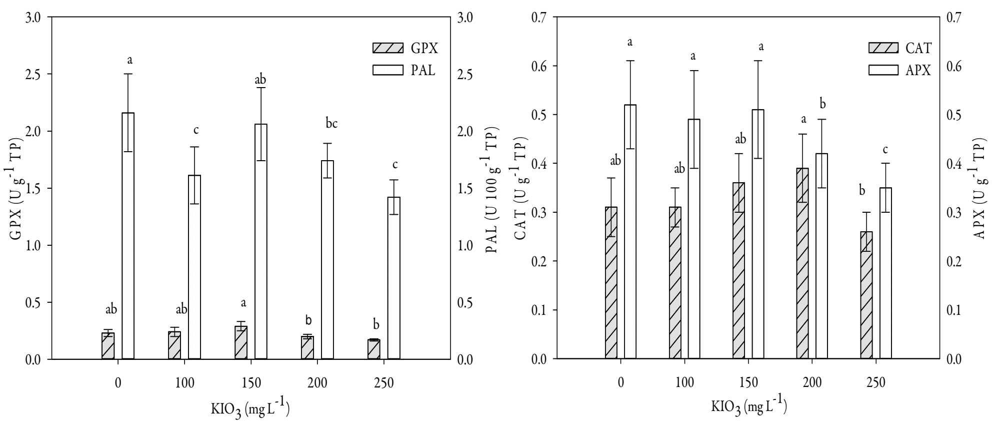

Figure 8. Effect of root imbibition by KIO_3 in the enzymatic compounds in tomato leaves
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Enzymatic compounds of tomato leaves by Na_2SeO_3 and KIO_3 interactions

The enzymatic activity in the tomato leaves significantly decreased as the concentrations of the treatments of the Na_2SeO_3 and KIO_3 Interactions were increased (Table 5).

Table 5. Effect of root imbibition by Na_2SeO_3 and KIO_3 interactions in the enzymatic compounds in tomato leaves

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})	PAL (U 100 g^{-1} TP)	GPX (U g^{-1} TP)	CAT (U g^{-1} TP)	APX (U g^{-1} TP)
0	0	4.6a	0.4abc	0.59abc	1.17a
0	100	3.7ab	0.5a	0.5abcd	1.17a
0	150	4.3a	0.4ab	0.68ab	1.17a
0	200	2.4cde	0.3abcd	0.84a	0.89b
0	250	2.6bc	0.2bcd	0.44bcde	0.69c
0,5	0	2.4cde	0.2abcd	0.38bcdef	0.51cde
0,5	100	1.1f	0.2bcd	0.29cdef	0.51cde
0,5	150	2.5bcd	0.3abcd	0.36bcdef	0.53cd
0,5	200	2.5bcd	0.2bcd	0.44bcde	0.5cde
0,5	250	0.9f	0.1bcd	0.37bcdef	0.4defg
1	0	0.9f	0.1bcd	0.33bcdef	0.44def
1	100	0.9f	0.1cd	0.37bcdef	0.34defgh
1	150	1.2ef	0.2bcd	0.46bcde	0.33defgh
1	200	1.4cdef	0.1cd	0.24cdef	0.32efgh
1	250	1.3def	0.1cd	0.23def	0.25gh
2	0	1.7cdef	0.1bcd	0.08f	0.28fgh
2	100	1.4cdef	0.1bcd	0.24cdef	0.26fgh
2	150	1.3def	0.1bcd	0.19def	0.32efgh
2	200	1.3def	0.1d	0.2def	0.23gh
2	250	1.3def	0.1cd	0.07f	0.21gh
3	0	1.0f	0.1d	0.19def	0.2h

3	100	0.8f	0.1d	0.15def	0.19h
3	150	0.8f	0.3abcd	0.1ef	0.18h
3	200	0.9f	0.1d	0.21def	0.17h
3	250	0.8f	0.1d	0.18def	0.2h

Different letters within the columns indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Antioxidant capacity of tomato fruits and leaves by Na_2SeO_3

The sodium selenite positively influenced the antioxidant capacity in tomato fruits. The hydrophilic compounds by ABTS and by DPPH radicals increased by 55,6 and 18,4%, respectively, in the dose of 3 mg L^{-1} in relation to the control treatments (Figure 9a). The lipophilic compounds by ABTS radical in tomato fruits were not significantly influenced by Na_2SeO_3.

The sodium selenite negatively influenced the antioxidant capacity in tomato leaves. The hydrophilic and lipophilic compounds by ABTS radical decreased by 35,1 and 66,7%, respectively, in the dose of 3 mg L^{-1} in relation to the control treatments (Figure 9b). The hydrophilic compounds by DPPH radical in tomato leaves were not significantly influenced by Na_2SeO_3. Saeedi *et al*. (2021) reported that the exogenous application of selenium presented favorable aspects in the antioxidant activity, as well as the improvement of secondary metabolites in cauliflower crop.

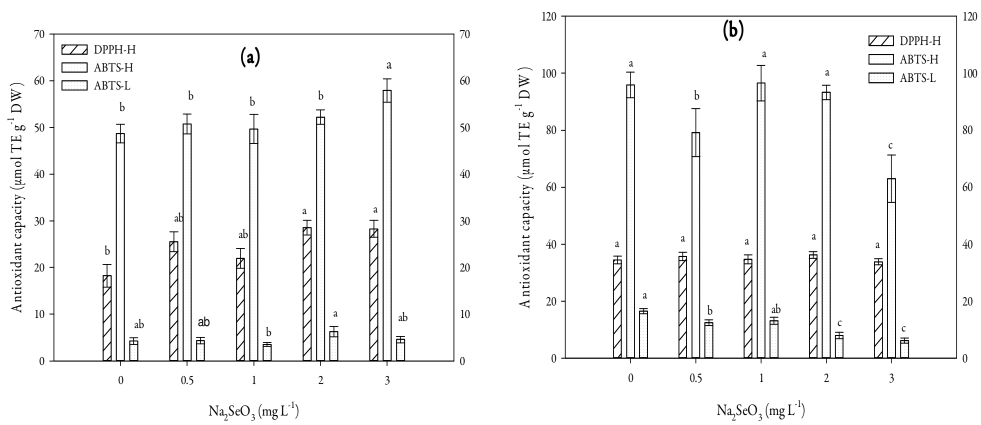

Figure 9. Effect of root imbibition by Na_2SeO_3 in the antioxidant capacity of tomato fruits and leaves
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Antioxidant capacity of tomato fruits and leaves by KIO_3

The antioxidant capacity of hydrophilic compounds by ABTS radical influenced by potassium iodate, significantly increased in the tomato fruits by 17,7% (Figure 10a) and significantly decreased in the tomato leaves by 20,5% (Figure 10b) both the two in the dose of 250 mg L^{-1} in relation to the control treatments. The lipophilic compounds by ABTS radical and the hydrophilic compounds by DPPH radical in tomato fruits and leaves were not significantly influenced by KIO_3. Medrano Macías *et al*. (2021) obtained statistical difference in the antioxidant capacity of strawberry with KIO_3 by the hydrophilic ABTS method, while the lipophilic ABTS and DPPH methods there were no effected. Smoleń *et al*. (2015) and Sarrou *et al*. (2019) reported that the use of KI and KIO_3 does not present an effect on the antioxidant capacity.

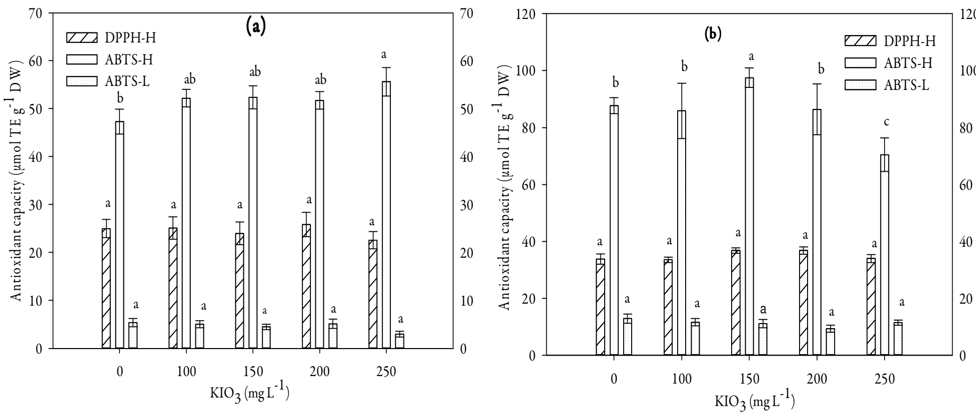

Figure 10. Effect of root imbibition by KIO_3 in the antioxidant capacity of tomato fruits and leaves
Different letters indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

Antioxidant capacity of tomato fruits and leaves by Na_2SeO_3 *and* KIO_3 *interactions*

The antioxidant capacity of the fruits by the hydrophilic ABTS method, presented differences where the treatment of 3-250 mg L^{-1}, presented a greater antioxidant capacity in comparison with the treatment of 1 mg L^{-1} of Na_2SeO_3, as well as with the control. The lipophilic ABTS method did not present an effect between the treatments. The DPPH method presented a negative effect in the treatments of 200 and 250 mg L^{-1} of KIO_3, which are the treatments with the lowest antioxidant capacity in tomato fruits (Table 6).

Table 6. Effect of root imbibition by Na_2SeO_3 and KIO_3 interactions in the antioxidant capacity (µmol TE g^{-1} DW) of tomato fruits and leaves

Na_2SeO_3 (mg L^{-1})	KIO_3 (mg L^{-1})	Fruto ABTS-H	Fruto ABTS-L	Fruto DPPH-H	Hoja ABTS-H	Hoja ABTS-L	Hoja DPPH-H
0	0	54.7bcdef	7.8ª	27.4ab	90.9cdef	20.4ª	34.9abcd
0	100	50.2cdef	2.0a	28.5ab	88.9cdef	17.0abc	33.0abcd
0	150	46.2defg	6.6ª	10.0b	81.6def	19.0ab	38.7abc
0	200	40.9efg	2.7ª	10.7b	131.4ab	11.5abcdef	30.4bcd
0	250	51.4cdef	1.9ª	14.3ab	86.5cdef	14.4abcdef	34.9abcd
0,5	0	40.2fg	3.0a	17.9ab	93.2cdef	12.4abcdef	35.8abc
0,5	100	56.9bcde	8.4ª	24.3ab	11.3g	15.7abcd	30.1bcd
0,5	150	51.5bcdef	3.9ª	27.3ab	111.1abc	11.9abcdef	40.6ab
0,5	200	62.7abc	3.7ª	37.0a	104.5bcd	14.2abcdef	43.1ª
0,5	250	42.1efg	2.4ª	20.8ab	75.4ef	7.7bcdef	28.7bcd
1	0	31.1g	2.8ª	15.8ab	70.4f	12.4abcdef	23.0d
1	100	42.0efg	3.0a	17.2ab	135.6ª	14.0abcdef	32.8abcd
1	150	67.9ab	4.4ª	25.5ab	105.7bc	14.6abcde	37.5abc
1	200	52.6bcdef	4.6ª	23.4ab	95.9cdef	11.9abcdef	40.5ab
1	250	54.3bcdef	2.7ª	27.7ab	74.9ef	12.9abcdef	39.4abc
2	0	59.0bcd	9.0a	33.8ª	101.6cde	9.9abcdef	37.1abc
2	100	53.1bcdef	4.7ª	29.3ab	100.8cde	7.8bcdef	37.2abc
2	150	45.6defg	3.0a	26.7ab	88.6cdef	6.8cdef	31.6abcd
2	200	50.6cdef	9.2ª	25.3ab	83.8cdef	3.5ef	36.7abc
2	250	52.4bcdef	5.1ª	27.4ab	91.3cdef	11.6abcdef	38.3abc
3	0	51.2cdef	3.9ª	29.9ab	82def	9.0abcdef	38.0abc
3	100	58.5bcd	6.7ª	26.0ab	92.3cdef	3.2ef	34.3abcd
3	150	50.3cdef	4.2ª	30.3ab	100.3cde	2.9f	35.2abc
3	200	51.7bcdef	5.2ª	32.5ab	16.1g	5.1def	33.0abcd
3	250	77.6ª	2.7ª	22.5ab	23.7g	10.4abcdef	28.2cd

Different letters within the columns indicate significant differences between treatments (Tukey HSD, $p \leq 0,05$). n = 4.

In the leaves, the antioxidant capacity presented an effect through hydrophilic ABTS, where the 1-100 mg L^{-1}, obtained a greater antioxidant capacity compared to the treatment of 0,5-100 mg L^{-1}, which was the treatment where there was a lower antioxidant activity in the leaves of the plant. In the lipophilic ABTS method, there was an effect in the control, where there was a higher antioxidant capacity compared to the treatment of 3-150 mg L^{-1}, on the other hand, by means of hydrophilic DPPH, an effect was presented in the 0,5-200 mg L^{-1} treatment, where a greater antioxidant capacity was obtained by this method, while the antioxidant capacity was affected with the use of 1 mg L^{-1} of Na_2SeO_3 (Table 6).

Conclusions

The application of KIO_3 and Na_2SeO_3 in root imbibition in tomato cultivation presents important aspects in the increase of non-enzymatic compounds such as vitamin C, phenols, flavonoids and reduced glutathione, as well as in antioxidant capacity; however, this method presented an inhibition in the evaluated antioxidant enzymes.

Authors' Contributions

Conceptualization: FMR and AMM; Data curation: FMR and MIPL; Formal analysis: ABM and AMM; Methodology: FMR and AMM; Resources: AMM, ABM and SGM; Software: FMR and MIPL; Supervision: AJM, ABMD and FMLV; Visualization: AJM, ABMD and FMLV; Writing - original draft: FMR, MIPL and AMM; Writing - review and editing: FMR, MIPL and AMM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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