

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO  
SUBDIRECCIÓN DE POSTGRADO



EFFECTO EN LA CALIDAD NUTRICIONAL DEL FRUTO DE TOMATE (*Solanum lycopersicum* L.) CON APLICACIONES DE YODO (I) Y SELENIO (Se)

Tesis

Que presenta FERNANDO MEJÍA RAMÍREZ

como requisito parcial para obtener el Grado de  
DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

Saltillo, Coahuila

Junio 2023

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Tesis

Elaborada por FERNANDO MEJÍA RAMÍREZ como requisito parcial para obtener el grado de Doctor en Ciencias en Agricultura Protegida con la supervisión y aprobación del Comité de Asesoría

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Saltillo, Coahuila

Junio 2023

## **AGRADECIMIENTOS**

A mis padres, Francisca Ramírez Azpeitia y Marco Antonio Mejía Camargo por el apoyo y la confianza incondicional que siempre me han brindado.

Al Dr. Álvaro Morelos Moreno por el tiempo dedicado y el apoyo a lo largo de este trayecto.

Al comité de asesoría: Dr. Francisco Marcelo Lara viveros, Dr. Adalberto Benavides Mendoza, Dra. Susana Gonzales morales, Dr. Antonio Juárez Maldonado, Dra. América Berenice Morales Días, gracias por su apoyo.

Al Dr. Mepivoseth Castelán Estrada por lo conocimientos y consejos brindados, los cuales fueron de gran importancia para llevar a cabo este proyecto.

A mi prometida María Itzel Pérez León, gracias por todo el tiempo y comprensión que me has tenido, gracias por estar siempre a mi lado y por todo los buenos y malos momentos que hemos pasado junto, te quiero.

A Tomasa Quiterio Gutiérrez y Héctor Manuel Rodríguez Moran, gracias por su amistad, gracias por los consejos y apoyo que se brindaron durante este trayecto.

Al consejo nacional de humanidades, ciencias y tecnologías (CONAHCYT) por la beca otorgada.

A la Universidad Autónoma Agraria Antonio Narro por la oportunidad de realizar los estudios de doctorado.

A mis compañeros y amigos, gracias por su apoyo y amistad.


## **DEDICATORIA**

A mis padres, Francisca Ramírez Azpeitia y Marco Antonio Mejía Camargo, por darme la vida y guiarme día a día... por la confianza y apoyo que siempre me han brindado.

A María Itzel Pérez León, por el cariño, apoyo y comprensión durante este trayecto, gracias por todo, te quiero.



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> Authors: Fernando Mejía-Ramírez, Adalberto Benavides-Mendoza \*,

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## INTRODUCCIÓN

El consumo insuficiente de micronutrientes como el yodo (I) y selenio (Se) a través de los alimentos es limitado lo que puede causar una mala nutrición, y se ve reflejado en el desarrollo y crecimiento de los humanos, el yodo y selenio, son elementos que se requieren en pequeñas cantidades, pero son de gran importancia para el funcionamiento adecuado de la glándula tiroidea, se ha demostrado estar asociada a la carcinogénesis y su tratamiento en diversas líneas celulares, actuando como antioxidante y como antiproliferativo (Aceves *et al.*, 2005; Avery y Hoffmann, 2018; Guillin *et al.*, 2019).

La aplicación de elementos como el yodo y el selenio en las plantas de cultivo, con el fin de corregir la deficiencia de elementos esenciales en la dieta humana ha tomado importancia nivel mundial, además, el uso de estos elementos en la agricultura presenta aspectos interesantes, debido a que estos elementos en las plantas puede favorecer el crecimiento y el potencial de tolerancia al estrés, cuando se aplica en concentraciones bajas (Hasanuzzaman *et al.*, 2010).

El yodo y selenio juegan un papel importante en el beneficio a plantas de cultivo, el selenio es considerado un bioestimulante, presentando efectos positivos, en el crecimiento y desarrollo del cultivo, donde su uso en los cultivos presenta una disminución en el contenido de  $H_2O_2$  y  $O_2^-$  (Zhu *et al.*, 2017), lo que indica que el selenio tiene un efecto en la activación del sistema de defensa para controlar la producción y acumulación de especies reactivas de oxígeno (ERO), para esto el sistema de las células vegetales aumentan los niveles de metabolitos antioxidantes no enzimáticos, incluidos el glutatión, el ascorbato y el tocoferol, y una amplia red de antioxidantes enzimáticos, como las superóxido dismutasas, catalasas, ascorbato peroxidasas y glutatión reductasas, entre otros (Mittler *et al.*, 2004).

El uso de estos elementos se consideran elementos beneficiosos, mas no esenciales, sin embargo, el selenio es catalogado como un bioestimulante vegetal inorgánico, debido a que ha demostrado una mejora en la absorción de nutrientes, aumenta la tolerancia de las plantas al estrés y mejora la calidad del rendimiento de los cultivos (Dima *et al.*, 2020; Hasanuzzaman *et al.*, 2020; de Mello Prado, 2021) debido a que en bajas concentraciones pueden funcionar como señalizadores para mejorar el sistema de defensa de la planta lo

que se refleja en un incremento en el contenido de metabolitos secundarios; sin embargo, en altas concentraciones puede provocar daño oxidativo en los tejidos (Mittler, 2017; Abedi *et al.*, 2021). Por su parte el tomate (*Solanum lycopersicum* L.) es uno de los cultivos de mayor importancia en México y el mundo, tanto por su importancia económica como por ser un alimento rico en vitaminas, minerales y antioxidante, al ser consumidos como vegetales frescos, ya sea crudos o cocidos, son una fuente importante de carotenoides y otros nutrientes que promueven la salud en la dieta humana (Meng *et al.*, 2022). Por tal motivo se planteó el objetivo de evaluar el efecto de aplicación de yodo y selenio con aplicaciones en imbibición de semilla, imbibición de raíz y aspersion foliar sobre los indicadores nutraceuticos y de capacidad antioxidante en frutos y hojas de tomate.

## REVISIÓN DE LITERATURA

### Imbibición

El proceso de germinación se ve afectado por factores externos como el tiempo de germinación y la ausencia o presencia de luz, los cuales pueden ayudar o inhibir la germinación en relación con la reserva (contenido nutricional) dentro de la semilla. Durante la germinación, algunas reservas de la semilla se degradan y se utilizan para la respiración y la síntesis de nuevos constituyentes celulares para el embrión en desarrollo, lo que provoca cambios significativos en las características bioquímicas, nutricionales y sensoriales (Kim *et al.*, 2012).

La imbibición es un proceso efectivo y común, utilizado para mejorar la calidad nutricional, asegura una mayor germinación y uniforme, al reducir el tiempo de germinación, aumenta la activación de enzimas pre-germinativas, producción de metabolitos, repara el ADN dañado y regula la ósmosis (Damaris *et al.*, 2019). El “Seed priming” es considerada una técnica a base de agua que permite la hidratación controlada de la semilla para desencadenar el metabolismo pregerminativo, pero no permite que emerja la radícula (Dutta, 2018).

La germinación de semillas no latentes tiene lugar en tres etapas, cuando la semilla reúne condiciones de crecimiento favorables se inicia el proceso de la germinación dividida en tres fases (Khan *et al.*, 2022):

- (I) Imbibición: inicia con una gran absorción de agua por parte de la semilla seca, lo que permite que las células de la semilla se hidraten, la semilla absorbe el máximo de agua para activar la vía de señalización del ácido giberélico (GA) y los factores de transcripción de las enzimas hidrolíticas, como la  $\alpha$ -amilasa que inician la descomposición del almidón en azúcares simples. Ambos procesos promueven la descomposición de los materiales del endospermo y proporcionan azúcar soluble para iniciar el proceso de germinación.
- (II) Fase de latencia: En esta fase existe un incremento en el contenido de ADN, síntesis de proteínas donde participan los ARNm.

(III) Protrusión de la radícula a través de la cubierta seminal a lo que llamamos una semilla germinada.

La absorción de agua durante la etapa de preparación de semillas es fundamental para la germinación y crecimiento de plántulas, y estas características tienen implicaciones agronómicas prácticas, al mejorar la germinación, velocidad de germinación, vigor en plántulas, longitud de raíces y el contenido de materia seca, eficiencia fotosintética, además de mejorar el estado bioquímico de la planta, al presentar una mayor actividad de la  $\alpha$ -amilasa y el contenido de carbohidratos solubles durante la germinación de la semilla (Siddique y Bose, 2007; Bose *et al.*, 2018; Marthandan *et al.*, 2020). El proceso de germinación da como resultado una serie de cambios bioquímicos en el interior de la semilla y la acumulación de diversos fitoquímicos (Cáceres *et al.*, 2014).

La imbibición de semillas actúa como una exposición al estrés previa a la germinación y crea una "memoria de estrés" en las células que facilita la expresión de respuestas de estrés en exposiciones posteriores (Paparella *et al.*, 2015), desde este punto de vista, la imbibición es similar al fenómeno de aclimatación durante la imbibición temprana de semillas (Amooaghaie y Tabatabaie, 2017)

La imbibición con nutrientes se ha propuesto como una técnica novedosa que combina los efectos positivos de la imbibición de semillas con un suministro mejorado de nutrientes. Las semillas se pre-tratan en soluciones que contienen los nutrientes limitantes en lugar de remojarlas solo en agua (Arif, 2005). La creciente evidencia sugiere que el estado de los nutrientes minerales de las plantas juega un papel crítico en el aumento de la resistencia de las plantas a los factores de estrés ambiental (Jisha *et al.*, 2013).

Existen diferentes técnicas de imbibición de semillas, como es el hidropriming, el cual consiste en poner la semillas en una solución a base agua pura, por su parte el osmopriming consiste en someter a las semillas en una solución osmótica, donde se han utilizado amplios compuestos como NaCl, KCl, KNO<sub>3</sub>, K<sub>3</sub>PO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub> y CaCl<sub>2</sub>, donde se ha establecido que dicha técnica promueve la tolerancia a la salinidad, aumento de la productividad, germinación, mejora la calidad de la semilla, reduce las aplicaciones de los

fertilizantes, inducción a resistencia de enfermedades y tolerancia a sequía (Singh *et al.*, 2020).

El uso de tratamientos de imbibición u osmopriming, se ha establecido como un método efectivo para mejorar la respuesta de las plantas a condiciones de estrés biótico y abiótico, mediante la alteración del metabolismo antioxidante (Kumar *et al.*, 2016), estimula el sistema antioxidante al mejorar la actividad enzimática como: superóxido dismutasa (SOD), ascorbato peroxidasa (APX), catalasa (CAT), y compuestos no enzimático como: glutatión reducido (GSH), ácido ascórbico (AsA) y compuestos fenólicos (Anaytullah y Bose, 2012; Paparella *et al.*, 2015). Los sistemas antioxidantes de las plantas eliminan la producción excesiva de especies reactivas de oxígeno (ERO) provocada por diversos tipos de estrés y desempeñan un papel importante durante el almacenamiento, la germinación y desarrollo de las semillas, este sistema antioxidante está compuesto tanto enzimáticos como no enzimáticos, donde sirven a la planta para reducir el nivel de las ERO, donde la catalasa degrada el  $H_2O_2$  en oxígeno y agua, mientras que el ascorbato peroxidasa utiliza el ácido ascórbico como donante de electrones para estimular la degradación del  $H_2O_2$ , mientras que el glutatión reducido es responsable de la producción del ácido ascórbico (Hussain *et al.*, 2019). El glutatión reductasa cataliza la regeneración de glutatión reducido (GSH) a partir de glutatión oxidado (GSSG), con NADPH como agente reductor. GSH elimina  $H_2O_2$  al reaccionar de forma no enzimática con  $O_2^-$ ,  $OH^-$ , así mismo el GSH, tiene la capacidad de reponer ácido ascórbico, a través del ciclo de ascorbato-glutation, el cual es de gran importancia para el sistema antioxidante (Millar *et al.*, 2003)

### **Elementos esenciales**

Hasta el momento, se ha demostrado que 16 elementos son esenciales para el crecimiento y el desarrollo de las plantas superiores según los criterios de esencialidad establecidos por (Arnon y Stout, 1939)

Criterios de esencialidad:

- La deficiencia de algún elemento, imposibilita que la planta complete su etapa vegetativa y reproductiva en su ciclo de vida
- La deficiencia de dicho elemento puede prevenirse o corregirse únicamente con el suministro de dicho elemento



- El elemento debe estar involucrado directamente en el metabolismo de la planta

Sin embargo, el uso de elementos como el yodo (I) y selenio (Se), son considerados elementos beneficiosos, mas no esenciales, El selenio es catalogado como un bioestimulante vegetal inorgánico, debido a que ha demostrado una mejora en la absorción de nutrientes, aumenta la tolerancia de las plantas al estrés y mejora la calidad del rendimiento de los cultivos (Dima *et al.*, 2020). Las plantas tienen la capacidad de absorber elementos químicos en el suelo o la solución de nutritiva con poca restricción, que podría ser un nutriente, nutrientes no minerales de la atmósfera o un elemento beneficioso y/o tóxico (Kathpalia y Bhatla, 2018), los elementos benéficos podrían ser considerados esenciales. Asimismo, algunos de los elementos benéficos son esenciales para ciertas familias o grupos de plantas, en esta clasificación se encuentra al aluminio (Al), cesio (Ce), cobalto (Co), lantano (La), sodio (Na), selenio (Se), silicio (Si), titanio (Ti), vanadio (V) y yodo (I) (Trejo-Téllez *et al.*, 2007)

Además de los elementos considerados esenciales para la vida vegetal, existen elementos considerados beneficiosos y también un grupo de elementos tóxicos. El elemento benéfico se define como aquel que estimula el crecimiento de las plantas, pero no es esencial o es esencial solo para ciertas especies o bajo ciertas condiciones (de Mello Prado, 2021). El silicio, el cobalto y el selenio se consideran beneficiosos para el crecimiento de ciertas plantas. (Trejo-Téllez *et al.*, 2007). Por su parte el yodo no se ha demostrado como un elemento esencial para las plantas terrestres (Cakmak *et al.*, 2017), sin embargo, se sabe que las plantas lo absorben por las raíces y las hojas y lo disipan a la atmósfera en forma gaseosa utilizando metiltransferasas de halógeno no dependientes del vanadio (Landini, 2011), por lo que es posible que el yodo realice funciones metabólicas aún no bien comprendidas en las plantas terrestres (Gonzali *et al.*, 2017)

### **Selenio**

El selenio (Se) es un micronutriente esencial para animales y humanos, aún no se ha establecido su esencialidad en las plantas. Sin embargo, juega un papel importante con beneficio en plantas de cultivo, particularmente bajo estrés biótico y abiótico. El selenio adopta vías de absorción y transporte en plantas similares a las del sulfato y/o fosfatos; puede reemplazar el azufre a nivel celular para sintetizar macromoléculas importantes,

incluidos aminoácidos, proteínas estructurales y funcionales específicas y/o proteínas no específicas potencialmente tóxicas y otros seleno-compuestos (Sarwar *et al.*, 2020).

Las raíces de las plantas adquieren el selenio de la solución de la rizosfera, se pueden absorber como selenato ( $\text{SeO}_4^{2-}$ ), selenito ( $\text{SeO}_3^{2-}$ ) y compuestos de organoselenio, como seleniocisteína (SeCys) y seleniometionina (SeMet), pero no se pueden absorber seleniuros o Se elemental (White, 2018). Las plantas son capaces de absorber iones de selenato y selenito en la raíz; sin embargo, ninguno de los iones se absorbe a través de un transportador específico de Se. El selenato se absorbe a través de los simportadores  $\text{H}^+$ /sulfato, los SULTR 1;1 y SULTR 1;2, son transportadores de alta afinidad, funcionan para absorber el sulfato en la raíz, sin embargo, se ha demostrado que ambos transportadores son capaces de transportar selenato, mientras que el selenito es absorbido por los transportadores de fosfato inorgánico (Pi) y las acuaporinas (Schiavon y Pilon-Smits, 2017; White, 2018; Trippe y Pilon-Smits, 2021)

La comprensión actual del metabolismo del Se en las angiospermas se basa en gran medida en el conocimiento del metabolismo del azufre (S) (White, 2016; Schiavon y Pilon-Smits, 2017). Los aspectos del metabolismo del Se ocurren tanto en el citosol como en los plástidos de las células de las hojas. Se cree que la mayor parte del selenato ingresa al citosol de las células de la hoja a través de transportadores SULTR en su membrana plasmática y luego es transportado al plástido por homólogos del transportador SULTR 3;1, que se encuentra en la membrana del cloroplasto (Cao *et al.*, 2013).

La base bioquímica de la toxicidad del Se radica en el hecho de que el Se y el S son análogos químicos. Por lo tanto, selenato y selenita se asimilan a SeCys y SeMet. La sustitución de cisteína y metionina en las proteínas por SeCys y SeMet que puede provocar la pérdida de la funcionalidad de las proteínas, que a su vez, se atribuye a proteínas mal plegadas o malformadas (Van Hoewyk, 2013). Además, la interacción del Se con las proteínas puede conducir a la formación de selenotrisulfuros, enlaces selenilsulfuro y diselenuros (Ganther, 1999).

El selenio mejora el crecimiento de las plantas y el potencial de tolerancia al estrés de las plantas cuando se aplica en concentraciones más bajas (Hasanuzzaman *et al.*, 2010). Asimismo, la preparación con selenio protege a las plantas del daño oxidativo

inducido por la temperatura al estimular el sistema de defensa antioxidante de las plantas (Chen y Sung, 2001), así mismo el uso de yodo y selenio en la imbibición de semillas, resulta un método viable, debido a que se puede llevar a cabo el enriquecimiento de los brotes con el uso de ambos elementos, sin embargo, la captación depende de la forma y/o combinaciones de los elementos (Jerše *et al.*, 2017)

## **Yodo**

Las plantas pueden absorber el yodo del suelo, donde las principales especies de yodo son; iones de yodo orgánico, yodato ( $\text{IO}_3$ ) y yoduro ( $\text{I}^-$ ), por su parte en la atmósfera se puede encontrar en forma gaseosa, en forma de yodo molecular ( $\text{I}_2$ ) y el yoduro de metilo ( $\text{CH}_3\text{I}$ ) (Medrano-Macías *et al.*, 2016). Las plantas pueden absorber yodo del suelo a través de las raíces y de la atmósfera por medio de las hojas. El yodato puede reducirse a yoduro por reductasas específicas identificadas en las raíces (Kato *et al.*, 2013) o, posiblemente por otras reductasas de plantas que usan  $\text{IO}_3$  como sustrato alternativo.

Una vez dentro de la planta, el yodo se mueve principalmente por la ruta xilemática. La volatilización y emisión de yodo a la atmósfera como yoduro de metilo, se produce a través de una enzima haluro metiltransferasa (HMT) dependiente de adenosilmetionina S (Redeker *et al.*, 2000; Itoh *et al.*, 2009; Medrano-Macías *et al.*, 2016). Se han elaborado algunas hipótesis con respecto a la absorción de yodo por la raíz y la subsiguiente carga del xilema en función de las afinidades químicas con otros halógenos, en particular el cloro u otros nutrientes (White y Broadley, 2009). Sin embargo, hasta la fecha no se han identificado transportadores de yodo en las plantas y no se conocen con precisión las formas de yodo que se mueven dentro de la planta ni las que se almacenan dentro de los tejidos.

Se sabe muy poco sobre la absorción y el transporte de yodo en las células vegetales, especialmente en el caso de la absorción de yodo en la hoja. Se sabe que el nitrato y el yodato son químicamente similares, y por lo tanto, pueden competir durante la absorción y el transporte en los sistemas biológicos (Cakmak *et al.*, 2017). El nitrato y el yodato posiblemente comparten la misma proteína transportadora, lo que puede causar una inhibición competitiva de la absorción de yodato en las especies de fitoplancton marino si el nivel de nitrato es alto en el medio (De La Cuesta y Manley, 2009).

El yodo en la planta presenta un incremento la producción de biomasa, la floración y también está presente en proteínas de raíces y brotes, y ayuda a modular la expresión de genes implicados en respuestas de defensa (Nascimento *et al.*, 2022) además, se ha demostrado que el selenio (Se) y el vanadio promueven la absorción de yodo en las plantas (Smoleń *et al.*, 2021).

## PRIMER ARTÍCULO

Seed priming based on iodine and selenium influences the nutraceutical compounds in tomato (*Solanum lycopersicum* L.) crop.



Article

# Seed Priming Based on Iodine and Selenium Influences the Nutraceutical Compounds in Tomato (*Solanum lycopersicum* L.) Crop

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**Citation:** Mejía-Ramírez, F.; Benavides-Mendoza, A.; González-Morales, S.; Juárez-Maldonado, A.; Lara-Viveros, F.M.; Morales-Díaz, A.B.; Morelos-Moreno, Á. Seed Priming Based on Iodine and Selenium Influences the Nutraceutical Compounds in Tomato (*Solanum lycopersicum* L.) Crop. *Antioxidants* **2023**, *12*, 1265. <https://doi.org/10.3390/antiox12061265>

Academic Editor: Evangelos Zoidis

Received: 21 May 2023

Revised: 2 June 2023

Accepted: 4 June 2023

Published: 13 June 2023



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**Abstract:** The use of trace elements in agriculture as a complement to crop fertilization programs is a practice that is gaining importance and relevance worldwide. Iodine and selenium perform essential functions in human health, related to the proper functioning of the thyroid gland, acting as antioxidants and antiproliferatives, and their limited intake through food consumption can cause malnutrition, reflected in the abnormal development and growth of humans. This research aimed to evaluate the nutraceutical quality of tomato (*Solanum lycopersicum* L.) in response to seed priming based on  $KIO_3$  (0, 100, 150, 200, 250  $mg\ L^{-1}$ ) and  $Na_2SeO_3$  (0, 0.5, 1, 2, 3  $mg\ L^{-1}$ ), performed by interaction from a  $5^2$ -factorial design and by independent factors in a 24-h imbibition time. The tomato crop was established under greenhouse conditions in 10-L polyethylene containers containing peat moss and perlite 1:1 (v/v). Regarding non-enzymatic antioxidant compounds, lycopene,  $\beta$ -carotene and flavonoid contents in tomato fruits significantly increased with  $KIO_3$  and  $Na_2SeO_3$  treatments; however, vitamin C content was negatively affected.  $KIO_3$  increased the phenol and chlorophyll-*a* contents of leaves. In relation to enzymatic activity,  $KIO_3$  positively influenced GSH content and PAL activity in tomato fruits.  $KIO_3$  also positively influenced GSH content in leaves while negatively affecting PAL and APX activities.  $Na_2SeO_3$  favored GSH content and GPX activity in tomato fruits and leaves.  $Na_2SeO_3$  negatively affected the antioxidant capacity of hydrophilic compounds by ABTS in fruits and leaves and favored hydrophilic compounds by DPPH in leaves. Seed imbibition based on  $KIO_3$  and  $Na_2SeO_3$  is a method that is implemented in the tomato crop and presents interesting aspects that favor the nutraceutical quality of tomato fruits, which may contribute to increasing the intake of these minerals in humans through tomato consumption.

**Keywords:**  $KIO_3$ ;  $Na_2SeO_3$ ; antioxidant; seed imbibition; tomato

## 1. Introduction

Seed imbibition is considered a water-based technique that allows controlled hydration of the seed to trigger pregerminative metabolism but does not allow radicle emergence [1]. Seed imbibition is a common and effective process used to improve nutritional quality. The germination process is affected by external factors such as temperature, relative humidity, and light conditions, which can help or inhibit germination in relation to the nutritional reserve within the seed. During the germination process, some seed reserves are degraded

and used for respiration and the synthesis of new cellular constituents for embryonic development, which causes changes in biochemical and nutritional characteristics [2].

The seed imbibition process consists of dipping the seeds in a water solution for some time, or imbibition time, allowing partial hydration without the radicle emerging. When a dry seed is kept in water, the germination process of non-dormant seeds occurs in three phases: imbibition, the lag phase, and protrusion of the radicle through the testa [3,4]. Seed priming is an effective method to improve seed physiological quality through uniformity and germination percentage [5]. It stimulates pre-germination metabolic processes, reduces the physical resistance of the endosperm during imbibition, repairs membranes, influences the development of immature embryos, and leaches inhibitors [3,6]. Seedlings from seed imbibition emerge faster, grow more vigorously, and tolerate adverse conditions (Table S1) related to certain physiological, biochemical, cellular, and molecular changes [7–9].

Treatments based on seed priming have been established as an effective method to improve the response of plants to biotic and abiotic stress conditions through the alteration of antioxidant metabolism [10]. This technique also stimulates the antioxidant system by improving the activity of antioxidant enzymes [11,12]. The use of iodine (I) and selenium (Se) in agriculture has become very relevant and important worldwide. At low concentrations, these elements present stimulatory effects and can favor the growth and development of crops and increase the content of secondary metabolites and antioxidant enzymes; however, at high concentrations, these elements can cause toxicity to plants.

Se plays an important role in the benefit of crop plants and is considered a biostimulant that promotes positive effects on the growth and development of crops. Se in the plants influences the accumulation of enzymes such as superoxide dismutases, catalases, ascorbate peroxidases, and glutathione reductases [13,14], which control the antioxidant metabolites, the production, and accumulation of reactive oxygen species (ROS), and activate the defense system [13]. Iodine and selenium are highly important in human health. The intake of these trace elements through food consumption is limited, which can cause malnutrition reflected in the abnormal development and growth of humans. These elements, required in small quantities, fulfill essential functions in the proper functioning of the thyroid gland (Table S2), which has recently been shown to be associated with carcinogenesis and its treatment in various cell lines, acting as an antioxidant and as an antiproliferative [15–17].

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 nutraceutical quality of tomato fruits and leaves.

## 2. Materials and Methods

### 2.1. Crop Establishment

The tomato crop was established in a tunnel-type greenhouse with a 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 environmental conditions in the greenhouse during the crop cycle were: air temperature of 20 °C, relative humidity of 34%, solar radiation of 735 W m<sup>-2</sup> and photosynthetically active radiation of 568 μmol m<sup>-2</sup> s<sup>-1</sup>. Potassium iodate (KIO<sub>3</sub>) (99%, Sigma Aldrich, St. Louis, MO, USA) and sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) (99%, Sigma Aldrich, St. Louis, MO, USA) were used as I and Se species, respectively. Saladette-type CID F1 (Harris Moran®, Davis, CA, USA) tomato seeds were primed in KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> solutions, and the seeds of the control treatment were primed in distilled water (Table 1) for a 24-h imbibition time (Figure S1). Then, the seeds were dried at room temperature for 48 h.



**Table 1.** Priming treatments in tomato seeds based on I and Se.

Variation Factor	Concentration (mg L <sup>-1</sup> )
KIO <sub>3</sub>	0, 100, 150, 200, 250
Na <sub>2</sub> SeO <sub>3</sub>	0, 0.5, 1, 2, 3

25 treatments (5<sup>2</sup> factorial), n = 4 replications, 100 experimental units.

#### 2.1.1. Preparation of KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> Treatments

A stock solution of potassium iodate (KIO<sub>3</sub>) at a concentration of 1000 ppm was prepared. A mass of 168.59 mg of KIO<sub>3</sub> was gauged to 100 mL with distilled water. Dilutions of 2.5, 3.75, 5, and 6.25 mL of the stock solution were gauged to 25 mL with distilled water to obtain the treatments of 100, 150, 200, and 250 mg L<sup>-1</sup>, respectively.

A stock solution of sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) at a concentration of 1000 ppm was prepared. A mass of 21.89 mg of Na<sub>2</sub>SeO<sub>3</sub> was gauged to 100 mL with distilled water. Dilutions of 12.5, 25, 50, and 75 µL of the stock solution were gauged to 25 mL with distilled water to obtain the treatments of 0.5, 1, 2, and 3 mg L<sup>-1</sup>, respectively.

#### 2.1.2. Sowing and Planting

After the seed priming process, seeds were sown in polystyrene trays with peat moss and perlite 1:1 (*v/v*) as substrates. Seedlings were planted 35 days after sowing in 10-L plastic containers with peat moss and perlite 1:1 (*v/v*) as substrate. Tomato plants were arranged in the greenhouse in a randomized complete block design with a factorial arrangement of two factors (KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub>) and five levels (concentrations in mg L<sup>-1</sup>) (Table 1). Fertilization consisted of Steiner-type nutrient solutions [18], diluted in drip irrigation. The same substrate and fertigation conditions were used in the control and KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> treatments to avoid another variation factor affecting the performance of the treatment.

#### 2.1.3. Sampling

Samples of the 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 [19]. 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.

### 2.2. Non-Enzymatic Compounds

#### 2.2.1. Vitamin C

The vitamin C content was determined by the 2,6-dichlorophenolindophenol titration method [20]. Here, 20 g of fresh fruit tissue was macerated by using a mortar and pestle with 10 mL of hydrochloric acid (2%), and 100 mL of distilled water was added and filtered with sterile gauze. A 10-mL aliquot was taken and titrated with 2,6-dichlorophenolindophenol until a pinkish color was obtained (Equation (1)). The results were expressed in mg per 100 g of fresh tissue (mg 100 g<sup>-1</sup> FW).

$$\text{Vitamin C} = \frac{\text{mL of 2,6-dichlorophenolindophenol} \times 0.088 \times \text{total volume} \times 100}{\text{aliquot volume} \times \text{sample weight}} \quad (1)$$

#### 2.2.2. Total Phenols

Total phenolic compounds were determined using the Folin–Ciocalteu method from the extraction with water:acetone [21], made by adding 50 µL of the extract, 200 µL folin–ciocalteu reagent, 500 µL Na<sub>2</sub>CO<sub>3</sub>, and 5 mL distilled water to a test tube. The mixture was vortexed for 30 s and placed in a water bath at 45 °C for 30 min. The absorbance of the



mixture was read at a 750 nm wavelength. The results were expressed in mg of gallic acid equivalents per gram of dry tissue ( $\text{mg GAE g}^{-1}$  DW).

#### 2.2.3. Total Flavonoids

Flavonoids were determined by the Dowd method, adapted by Arvouet-Grand et al. [22]. It was made by adding 2 mL of the extract and 2 mL of  $\text{AlCl}_3$  (2%) methanolic solution to a test tube. The mixture was allowed to react for 20 min in the dark. The absorbance of the mixture was read at a 415 nm wavelength. The results were expressed in mg of quercetin equivalents per 100 g of dry tissue ( $\text{mg QE } 100 \text{ g}^{-1}$  DW).

#### 2.2.4. Chlorophyll

Chlorophyll content was quantified using the method proposed by Munira et al. [23]. Here, 1 g of fresh plant material was homogenized, and then 5 mL acetone (90%) was added. Additionally, 10 mg of magnesium carbonate (to protect and stabilize chlorophylls) was added, 2 mL of the extract was centrifuged at  $9408 \times g$  for 5 min at  $4^\circ\text{C}$ , and the supernatant was extracted. Chlorophyll-*a* and chlorophyll-*b* were quantified by reading the absorbances at 663 and 645 nm wavelengths, respectively. The results were computed (Equations (2)–(4)) and expressed in  $\mu\text{g}$  per gram of fresh tissue ( $\mu\text{g g}^{-1}$  FW).

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

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

$$\text{Total chlorophyll} = 34.02 \times A_{645} + 18.8 \times A_{663} \quad (4)$$

#### 2.2.5. Lycopene and $\beta$ -carotene

Lycopene and  $\beta$ -carotene contents were determined according to Nagata and Yamashita [24]. Here, 100 mg of lyophilized tissue was mixed with 2 mL of hexane:acetone (3:2) solution. An aliquot was taken from the supernatant and the absorbances at 453, 505, 645, and 663 nm wavelengths were read. The results were computed (Equations (5) and (6)) and expressed in mg per 100 g of dry tissue ( $\text{mg } 100 \text{ g}^{-1}$  DW).

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

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

### 2.3. Enzymatic Activity

#### 2.3.1. Extraction

Samples of leaves and ripe 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  $14,700 \times g$  for 10 min at  $4^\circ\text{C}$ . The supernatant was collected and filtered with a 0.45-mm-diameter nylon membrane. Finally, the supernatant was diluted with phosphate buffer (1:20). 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.

#### 2.3.2. Reduced Glutathione (GSH)

GSH quantification was performed by the spectrophotometric technique [25]. It was made by adding 0.48 mL of the extract, 2.2 mL of  $\text{Na}_2\text{HPO}_4$  (0.32 M), and 0.32 mL of DTNB (1 mM) to a test tube. The sample was allowed to react for 15 min at room temperature,

and the absorbance was read at a 412 nm wavelength. 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 ( $mM\ GSHE\ mL^{-1}\ min^{-1}\ DW$ ).

#### 2.3.3. Glutathione Peroxidase (GPX) (QE 1.11.1.9)

GPX was determined using the Flohé and Günzler [26] method, adapted by Xue et al. [25]. It was performed by adding 0.2 mL of the extract, 0.5 mL of GSH (1 mM), and 0.2 mL of  $Na_2HPO_4$  (0.067 M) to a test tube. The sample was placed in a water bath at 25 °C for 5 min, and 0.2 mL of  $H_2O_2$  was added. The mixture was allowed to react for 10 min, and the reaction was stopped with 1 mL of trichloroacetic acid. The mixture was centrifuged in a C2500 refrigerated microcentrifuge (Labnet Prism®) at  $846\times g$  for 10 min at 4 °C. Then, 0.48 mL of the obtained mixture, 2.2 mL of  $Na_2HPO_4$  (0.32 M), and 0.32 mL of 5.5 dithionis-2 nitro benzoic acid dye (1 mM) were added to a test tube. The mixture absorbance was read at a 412 nm wavelength. 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 ( $mM\ GSHE\ mL^{-1}\ min^{-1}\ DW$ ).

#### 2.3.4. Phenylalanine Ammonium Lyase (PAL) (QE 4.3.1.5)

PAL was determined according to Sykłowska-Baranek et al. [27]. It was performed by adding 0.1 mL of the extract and 0.9 mL of L-phenylalanine (6 mM) to a test tube. The mixture was incubated in a water bath for 30 min at 40 °C, and 0.25 mL of HCl (5 N) was added. The sample was placed in an ice bath, and 5 mL of distilled water was added. The mixture absorbance was read at a 290 nm wavelength. The results were expressed in units per 100 g of total protein ( $U\ 100\ g^{-1}\ TP$ ), where U is equal to  $\mu mol$  of trans-cinnamic acid equivalents per mL per minute of dry tissue ( $\mu mol\ TCAE\ mL^{-1}\ min^{-1}\ DW$ ).

#### 2.3.5. Catalase (CAT) (QE 1.11.1.6)

CAT was determined by the spectrophotometric method [28], which is based on the quantification of the  $H_2O_2$  oxidation rate by absorbance difference ( $T_0 - T_1$ ).  $T_0$  was determined by adding 0.1 mL of the extract, 0.4 mL of  $H_2SO_4$  (5%), and 1 mL of  $H_2O_2$  (100 mM) to a test tube.  $T_1$  was computed by adding 0.1 mL of extract and 1 mL of  $H_2O_2$  (100 mM), stirring for 1 min, and adding 0.4 mL of  $H_2SO_4$  (5%). The mixture absorbance was read at a 270 nm wavelength. 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 ( $mM\ H_2O_2E\ mL^{-1}\ min^{-1}\ DW$ ).

#### 2.3.6. Ascorbate Peroxidase (APX) (EC 1.11.1.11)

APX quantification was performed according to the Nakano and Asada [29] method, which is based on the quantification of the  $H_2O_2$  oxidation rate by absorbance difference ( $T_0 - T_1$ ).  $T_0$  was determined by adding 0.1 mL of the extract, 0.5 mL of ascorbate ( $10\ mg\ L^{-1}$ ), 0.4 mL of  $H_2SO_4$  (5%) to stop the reaction, and 1 mL of  $H_2O_2$  (100 mM) to a test tube.  $T_1$  was computed by adding 0.1 mL of extract and 1 mL of  $H_2O_2$  (100 mM) to a test tube, stirring for 1 min, and adding 0.4 mL of  $H_2SO_4$  (5%) to stop the reaction. The mixture absorbance was read at a 266 nm wavelength. The results were expressed in units per gram of total protein ( $U\ g^{-1}\ TP$ ), where U is equal to  $\mu mol$  of ascorbate oxidized equivalents per mL per minute of dry tissue ( $\mu mol\ AOE\ mL^{-1}\ min^{-1}\ DW$ ).

### 2.4. Antioxidant Capacity

#### 2.4.1. Antioxidant Capacity of 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 [30]. It is based on the discoloration of the ABTS radical cation. This radical was obtained from the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM) (1:1) under dark conditions at 25 °C for 16 h.

Subsequently, it was diluted with ethanol (20%) to obtain an absorbance of  $0.7 \pm 0.01$  at a 750 nm wavelength.

The antioxidant capacity of hydrophilic compounds by ABTS was determined by sample extraction performed with phosphate buffer, where 5  $\mu\text{L}$  of extract and 245  $\mu\text{L}$  of the dilution of ABTS radicals (7 mM) were placed in a microplate and allowed to react for 7 min in the dark. The absorbance of the extract was measured at a 750 nm wavelength.

The antioxidant capacity of lipophilic compounds as determined by ABTS was calculated by sample extraction performed with a hexane:acetone solution (3:2). Both the antioxidant capacity results of hydrophilic and lipophilic compounds by ABTS were expressed in  $\mu\text{mol}$  of Trolox equivalents per gram of dry tissue ( $\mu\text{mol TE g}^{-1} \text{ DW}$ ).

#### 2.4.2. Antioxidant Capacity of Hydrophilic Compounds by DPPH

Antioxidant capacity by DPPH radical (2,2-Diphenyl-1-picrylhydrazyl) was performed according to Brand-Williams et al. [31]. The stock solution was prepared by mixing 2.5 mg of the DPPH radical with 100 mL of methanol. The absorbance of the solution was adjusted to  $0.7 \pm 0.02$  at a 515 nm wavelength.

The antioxidant capacity of hydrophilic compounds by DPPH was determined as follows: 6  $\mu\text{L}$  of the obtained extract was taken with phosphate buffer, and 234  $\mu\text{L}$  of the DPPH radical was added. The decrease in absorbance of the extract after 30 min was measured at a 515 nm wavelength. The antioxidant capacity results of hydrophilic compounds by DPPH were expressed in  $\mu\text{mol}$  of Trolox equivalents per gram of dry tissue ( $\mu\text{mol TE g}^{-1} \text{ DW}$ ).

#### 2.4.3. Statistical Analyses

The results were analyzed 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<sup>®</sup> 2020e.

### 3. Results

#### 3.1. Non-Enzymatic Compounds in Tomato Fruits by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Interactions

The lycopene and  $\beta$ -carotene contents of tomato fruits were significantly modified by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions. The lycopene content increased 110.6% in the 200–2  $\text{mg L}^{-1}$  interaction and decreased 77.3% in the 200–3  $\text{mg L}^{-1}$  interaction in relation to the control treatment. The  $\beta$ -carotene content increased 157.1% in the 200–1  $\text{mg L}^{-1}$  interaction and decreased 90.5% in the 0–0.5  $\text{mg L}^{-1}$  interaction in relation to the control treatment (0–0  $\text{mg L}^{-1}$  interaction).

Phenol and flavonoid contents in tomato fruits significantly decreased in response to  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions. The phenol content increased by 9.7% in the 200–1  $\text{mg L}^{-1}$  interaction and decreased by 32.3% in the 150–1  $\text{mg L}^{-1}$  interaction in relation to the control treatment. Flavonoid content increased by 13.3% in the 250–1  $\text{mg L}^{-1}$  interaction and decreased by 35% in the 200–3  $\text{mg L}^{-1}$  interaction in relation to the control treatment.

The vitamin C content in tomato fruits was non-significantly influenced by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions (Table 2).

**Table 2.** Effect of seed priming based on  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions on the non-enzymatic antioxidant compounds in tomato fruits.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	Vitamin C ( $\text{mg } 100 \text{ g}^{-1} \text{ FW}$ )	Phenols ( $\text{mg GAE g}^{-1} \text{ DW}$ )	Flavonoids ( $\text{mg QE } 100 \text{ g}^{-1} \text{ DW}$ )	Lycopene ( $\text{mg } 100 \text{ g}^{-1} \text{ DW}$ )	$\beta$ -carotene ( $\text{mg } 100 \text{ g}^{-1} \text{ DW}$ )
0	0	18.8 <i>abcdef</i>	3.1 <i>ab</i>	18.0 <i>ab</i>	6.6 <i>cd</i>	2.1 <i>efg</i>
0	100	17.6 <i>bcdef</i>	2.9 <i>abc</i>	17.2 <i>abc</i>	12.4 <i>b</i>	3.8 <i>abcde</i>
0	150	21.5 <i>a</i>	2.7 <i>abc</i>	12.6 <i>fghi</i>	4.0 <i>efg</i>	1.9 <i>fgh</i>
0	200	20.5 <i>abc</i>	2.6 <i>abc</i>	15.8 <i>bcdef</i>	4.3 <i>efg</i>	1.6 <i>fgh</i>
0	250	18.0 <i>bcdef</i>	2.6 <i>abc</i>	12.4 <i>hi</i>	2.9 <i>gh</i>	1.5 <i>fgh</i>

Table 2. Cont.

Na <sub>2</sub> SeO <sub>3</sub> (mg L <sup>-1</sup> )	KIO <sub>3</sub> (mg L <sup>-1</sup> )	Vitamin C (mg 100 g <sup>-1</sup> FW)	Phenols (mg GAE g <sup>-1</sup> DW)	Flavonoids (mg QE 100 g <sup>-1</sup> DW)	Lycopene (mg 100 g <sup>-1</sup> DW)	β-carotene (mg 100 g <sup>-1</sup> DW)
0.5	0	19.2 abcdef	2.7 abc	13.4 defghi	4.3 efg	0.2 h
0.5	100	16.2 f	3.0 abc	15.4 bcdefghi	6.6 cd	2.1 efg
0.5	150	16.5 ef	2.6 abc	17.3 abc	13.8 a	3.9 abc
0.5	200	17.9 bcdef	2.8 abc	15.7 bcdefg	4.9 ef	0.8 gh
0.5	250	19.5 abcde	2.9 abc	18.0 ab	7.8 c	1.7 fgh
1	0	19.1 abcdef	2.8 abc	12.9 efghi	3.8 fg	2.0 fg
1	100	20.6 ab	2.7 abc	15.1 bcdefghi	6.4 cd	3.0 cdef
1	150	17.1 def	2.1 c	15.9 bcde	6.6 cd	4.2 abc
1	200	19.8 abcd	3.4 a	16.8 bc	11.6 b	5.4 a
1	250	19.07 abcdef	3.0 abc	20.4 a	6.9 c	4.1 abcd
2	0	19.3 abcde	2.3 bc	17.2 abc	3.0 gh	2.6 cdef
2	100	18.2 bcdef	2.7 abc	14.8 cdefghi	3.2 gh	2.7 cdef
2	150	18 bcdef	3.0 ab	17.5 abc	11.2 b	5.2 ab
2	200	16.8 def	3.0 abc	17.1 bc	13.9 a	4.9 ab
2	250	19.3 abcdef	2.9 abc	15.8 bcdef	6.4 cd	3.7 be
3	0	19.2 abcdef	2.6 abc	15.3 bcdefghi	2.3 hi	2.5 def
3	100	19.5 abcde	2.9 abc	16.2 bcd	7.5 c	3.8 abcde
3	150	17.4 cdef	2.8 abc	14.9 bcdefghi	5.4 de	3.8 abcd
3	200	18.8 abcdef	2.3 abc	11.7 i	1.5 i	1.7 fgh
3	250	18.9 abcdef	2.7 abc	12.5 ghi	3.1 gh	2.6 cdef

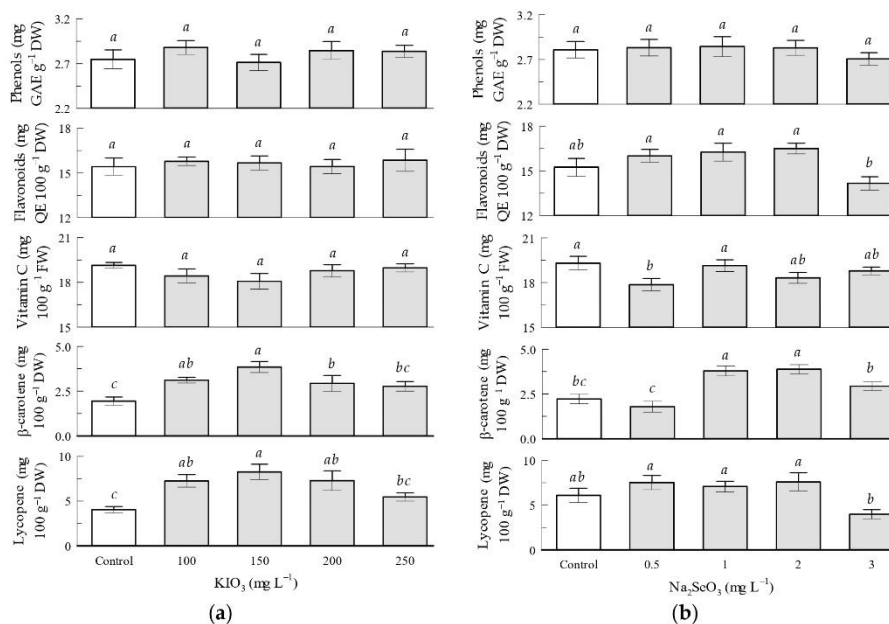
Different letters within the columns indicate significant differences between the treatment interactions (Tukey HSD,  $p \leq 0.05$ ),  $n = 4$ .

### 3.2. Non-Enzymatic Compounds in Tomato Fruits by KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> Factors

Regarding the potassium iodate factor in the tomato fruits, the vitamin C content significantly decreased by 5.2% with KIO<sub>3</sub> in the 150 mg L<sup>-1</sup> treatment in relation to the control treatment. Phenol and flavonoid contents were not influenced by KIO<sub>3</sub>. Lycopene and β-carotene contents significantly increased with all KIO<sub>3</sub> treatments, where the 150 mg L<sup>-1</sup> dose allowed reaching increments of 105 and 100% on lycopene and β-carotene contents, respectively, in relation to the control treatment, which contrasts with the results of vitamin C (Figure 1a).

Regarding the sodium selenite factor in the tomato fruits, the vitamin C content significantly increased by 9% with Na<sub>2</sub>SeO<sub>3</sub> in the 1 mg L<sup>-1</sup> treatment in relation to the 0.5 mg L<sup>-1</sup> treatment. The phenol content was not influenced by Na<sub>2</sub>SeO<sub>3</sub>. Flavonoid content increased by 8.6% with Na<sub>2</sub>SeO<sub>3</sub> in the 2 mg L<sup>-1</sup> treatment and decreased by 7.2% in the 3 mg L<sup>-1</sup> doses in relation to the control treatment. The lycopene content increased by 25% with Na<sub>2</sub>SeO<sub>3</sub> in the 0.5 and 2 mg L<sup>-1</sup> treatments and decreased by 35% in the 3 mg L<sup>-1</sup> treatment, in relation to the control treatment. The β-carotene content increased with Na<sub>2</sub>SeO<sub>3</sub> at 1–3 mg L<sup>-1</sup>, reaching the highest increase by 72.7% in the 2 mg L<sup>-1</sup> treatment in relation to the control treatment. The Na<sub>2</sub>SeO<sub>3</sub> treatment at 2 mg L<sup>-1</sup> presented the best performance because it resulted in the highest increases in lycopene, β-carotene, and flavonoid contents in tomato fruits in relation to the corresponding control treatment (Figure 1b).





**Figure 1.** Non-enzymatic antioxidant compounds in tomato fruits: (a) Seed priming based on  $\text{KIO}_3$ ; (b) Seed priming based on  $\text{Na}_2\text{SeO}_3$ . Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

### 3.3. Non-Enzymatic Compounds in Tomato Leaves by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Interactions

The phenol content was significantly influenced by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions, that is, increased by 31.6% in the 100–0.5  $\text{mg L}^{-1}$  interaction and decreased by 28.4% in the 0–0.5  $\text{mg L}^{-1}$  interaction in relation to the control treatment (0–0  $\text{mg L}^{-1}$  interaction). The flavonoid content significantly decreased by 21.4% in the 100–3  $\text{mg L}^{-1}$  interaction in relation to the control treatment. Chlorophyll and  $\beta$ -carotene were non-significantly influenced by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions in relation to the respective control treatments.

Chlorophyll-*a* and total chlorophyll significantly increased by 3.2% and 2.4%, respectively, in the 100–0  $\text{mg L}^{-1}$  interaction in relation to the 0–3  $\text{mg L}^{-1}$  interaction, that is, removing the  $\text{KIO}_3$  and raising the  $\text{Na}_2\text{SeO}_3$  doses (Table 3).

**Table 3.** Effect of seed priming based on  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions on the non-enzymatic antioxidant compounds in tomato leaves.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	Phenols ( $\text{mg GAE g}^{-1}$ DW)	Flavonoids ( $\text{mg QE } 100 \text{ g}^{-1}$ DW)	Chlorophyll <i>a</i> ( $\mu\text{g g}^{-1}$ FW)	Chlorophyll <i>b</i> ( $\mu\text{g g}^{-1}$ FW)	Total chl. ( $\mu\text{g g}^{-1}$ FW)	$\beta$ -carotene ( $\text{mg } 100 \text{ g}^{-1}$ DW)
0	0	9.5 cdef	60.4 ab	93.0 abc	81.3 a	174.7 ab	0.22 a
0	100	10.3 abcde	58.2 abc	94.4 a	81.0 a	175.8 a	0.15 a
0	150	10.5 abcde	52.8 abc	93.1 abc	81.3 a	174.7 ab	0.22 a
0	200	11.1 abcd	58.1 abc	92.8 abc	80.8 a	174.0 ab	0.25 a
0	250	8.5 efg	48.8 abc	91.9 bc	80.3 a	172.5 ab	0.23 a
0.5	0	6.8 g	51.4 abc	92.7 abc	80.6 a	173.6 ab	0.23 a
0.5	100	12.5 a	48.4 bc	94.0 ab	81.0 a	175.4 a	0.24 a
0.5	150	9.16 efg	55.4 abc	92.9 abc	81.3 a	174.5 ab	0.19 a
0.5	200	11.13 bcd	58.9 abc	92.3 abc	81.1 a	173.7 ab	0.24 a
0.5	250	9.4 def	56.8 abc	93.0 abc	81.4 a	174.7 ab	0.28 a

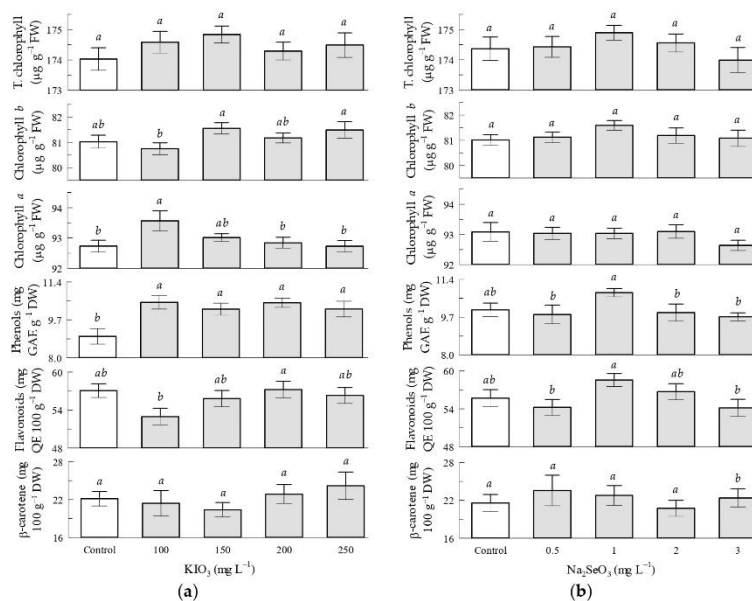
Table 3. Cont.

Na <sub>2</sub> SeO <sub>3</sub> (mg L <sup>-1</sup> )	KIO <sub>3</sub> (mg L <sup>-1</sup> )	Phenols (mg GAE g <sup>-1</sup> DW)	Flavonoids (mg QE 100 g <sup>-1</sup> DW)	Chlorophyll <i>a</i> (μg g <sup>-1</sup> FW)	Chlorophyll <i>b</i> (μg g <sup>-1</sup> FW)	Total chl. (μg g <sup>-1</sup> FW)	β-carotene (mg 100 g <sup>-1</sup> DW)
1	0	10.7 <i>abcde</i>	58.1 <i>abc</i>	92.9 <i>abc</i>	81.4 <i>a</i>	174.6 <i>ab</i>	0.23 <i>a</i>
1	100	10.1 <i>abcde</i>	54.2 <i>abc</i>	93.2 <i>abc</i>	80.6 <i>a</i>	174.1 <i>ab</i>	0.24 <i>a</i>
1	150	10.7 <i>abcde</i>	59.6 <i>abc</i>	92.8 <i>abc</i>	81.8 <i>a</i>	175.0 <i>ab</i>	0.21 <i>a</i>
1	200	10.5 <i>abcde</i>	61.0 <i>ab</i>	93.5 <i>abc</i>	81.8 <i>a</i>	175.7 <i>a</i>	0.21 <i>a</i>
1	250	11.8 <i>abc</i>	59.6 <i>abc</i>	92.5 <i>abc</i>	82.1 <i>a</i>	174.9 <i>ab</i>	0.25 <i>a</i>
2	0	7.6 <i>fg</i>	58.1 <i>abc</i>	93.3 <i>abc</i>	81.8 <i>a</i>	175.4 <i>a</i>	0.19 <i>a</i>
2	100	9.4 <i>cdef</i>	56.3 <i>abc</i>	93.6 <i>abc</i>	80.1 <i>a</i>	174.0 <i>ab</i>	0.18 <i>a</i>
2	150	11.0 <i>abcd</i>	50.4 <i>abc</i>	92.9 <i>abc</i>	81.0 <i>a</i>	174.1 <i>ab</i>	0.20 <i>a</i>
2	200	9.3 <i>def</i>	57.2 <i>abc</i>	92.9 <i>abc</i>	81.3 <i>a</i>	174.6 <i>ab</i>	0.21 <i>a</i>
2	250	12.1 <i>ab</i>	61.4 <i>a</i>	92.6 <i>abc</i>	81.6 <i>a</i>	174.5 <i>ab</i>	0.27 <i>a</i>
3	0	10.0 <i>bcdef</i>	57.1 <i>abc</i>	91.5 <i>c</i>	79.8 <i>a</i>	171.6 <i>b</i>	0.24 <i>a</i>
3	100	9.9 <i>bcdef</i>	47.5 <i>c</i>	92.4 <i>abc</i>	80.8 <i>a</i>	173.5 <i>ab</i>	0.26 <i>a</i>
3	150	9.3 <i>def</i>	60.7 <i>ab</i>	93.2 <i>abc</i>	82.2 <i>a</i>	175.7 <i>a</i>	0.20 <i>a</i>
3	200	10.2 <i>abcde</i>	50.7 <i>abc</i>	92.4 <i>abc</i>	80.6 <i>a</i>	173.3 <i>ab</i>	0.23 <i>a</i>
3	250	9.0 <i>defg</i>	54.7 <i>abc</i>	93.5 <i>abc</i>	81.9 <i>a</i>	175.6 <i>a</i>	0.18 <i>a</i>

Different letters within the columns indicate significant differences between the treatment interactions (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

### 3.4. Non-Enzymatic Compounds in Tomato Leaves by KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> Factors

Regarding the potassium iodate factor in the tomato leaves, the phenol content significantly increased with all KIO<sub>3</sub> treatments, where the 100 and 200 mg L<sup>-1</sup> treatments reached increments of 16.9% in relation to the control treatment. The chlorophyll-*a* content increased by 0.9% with KIO<sub>3</sub> in the 100 mg L<sup>-1</sup> treatment in relation to the control treatment. Flavonoid, chlorophyll-*b*, and β-carotene contents were not influenced by KIO<sub>3</sub> (Figure 2a).



**Figure 2.** Non-enzymatic antioxidant compounds in tomato leaves: (a) Seed priming based on KIO<sub>3</sub>; (b) Seed priming based on Na<sub>2</sub>SeO<sub>3</sub>. Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

Regarding the sodium selenite factor in the tomato leaves, all the non-enzymatic antioxidant compounds were not significantly influenced by  $\text{Na}_2\text{SeO}_3$  (Figure 2b).

### 3.5. Enzymatic Activity in Tomato Fruits by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Interactions

Regarding the tomato fruits, the (GSH) content and the PAL enzymatic activity were significantly increased by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions: GSH by 35% in the 150–0.5  $\text{mg L}^{-1}$  interaction and PAL by 441.7% in the 100–1  $\text{mg L}^{-1}$  interaction in relation to the respective control treatments (0–0  $\text{mg L}^{-1}$  interaction).

On the other hand, the enzymatic activities of GPX, CAT, and APX were not significantly influenced by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions in relation to the respective control treatments.

Lower and higher changes in GPX values were  $-30.6$  and  $238.9\%$  in the 100–2 and 0–3  $\text{mg L}^{-1}$  interactions, respectively, in relation to the corresponding control treatments. Lower and higher changes in CAT values were  $-14.8$  and  $70.4\%$  in the 0–3 and 100–3  $\text{mg L}^{-1}$  interactions, respectively, in relation to the corresponding control treatments. Lower and higher changes in APX values were  $-46.4$  and  $21.4\%$  in the 150–1 and 100–2  $\text{mg L}^{-1}$  interactions, respectively, in relation to the corresponding control treatments (Table 4).

**Table 4.** Effect of seed priming based on  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions on the enzymatic activity in tomato fruits.

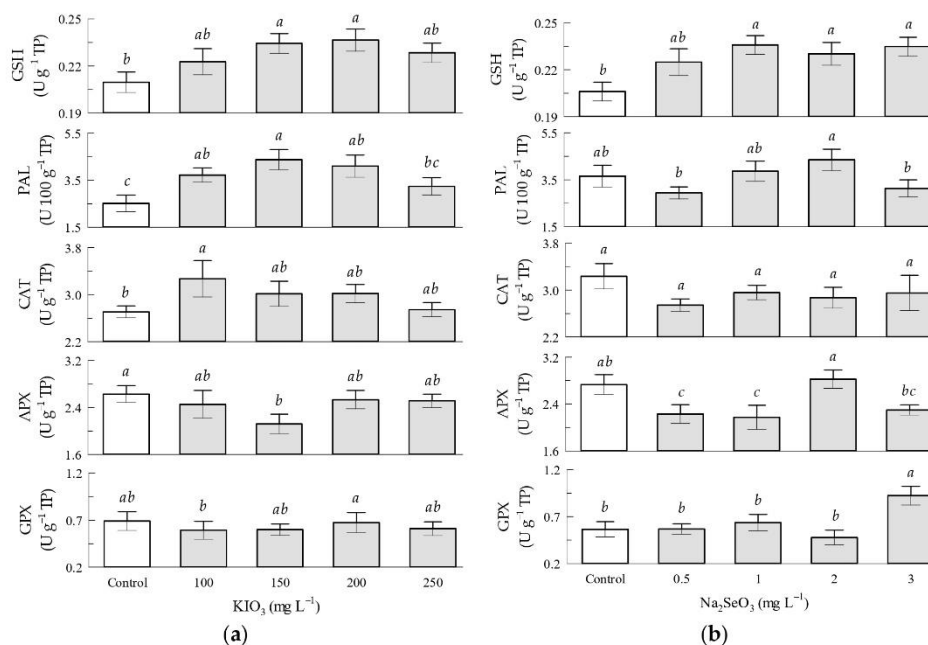
$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	GSH ( $\text{U g}^{-1}$ TP)	GPX ( $\text{U g}^{-1}$ TP)	PAL ( $\text{U } 100 \text{ g}^{-1}$ TP)	CAT ( $\text{U g}^{-1}$ TP)	APX ( $\text{U g}^{-1}$ TP)
0	0	0.20 bc	0.36 ab	1.2 b	2.7 ab	2.8 ab
0	100	0.22 abc	0.59 ab	4.1 ab	2.9 ab	2.3 ab
0	150	0.21 abc	0.54 ab	4.4 ab	4.0 ab	2.7 ab
0	200	0.20 abc	0.77 ab	4.7 ab	3.1 ab	2.9 ab
0	250	0.20 abc	0.57 ab	3.7 ab	3.2 ab	2.7 ab
0.5	0	0.20 bc	0.47 ab	3.0 ab	2.6 ab	3.0 ab
0.5	100	0.20 abc	0.48 ab	3.6 ab	2.6 ab	1.9 ab
0.5	150	0.27 a	0.71 ab	3.8 ab	2.9 ab	1.6 ab
0.5	200	0.25 abc	0.72 ab	2.3 ab	3.0 ab	2.2 ab
0.5	250	0.21 abc	0.46 ab	1.7 b	2.5 ab	2.3 ab
1	0	0.20 abc	0.73 ab	3.0 ab	3.0 ab	2.0 ab
1	100	0.25 abc	0.43 ab	2.3 ab	2.8 ab	2.4 ab
1	150	0.22 abc	0.72 ab	4.1 ab	3.0 ab	1.5 b
1	200	0.26 a	0.70 ab	6.5 a	3.4 ab	2.4 ab
1	250	0.25 abc	0.60 ab	3.2 ab	2.4 ab	2.3 ab
2	0	0.21 abc	0.68 ab	2.4 ab	2.7 ab	2.8 ab
2	100	0.19 c	0.25 b	5.0 ab	3.3 ab	3.4 a
2	150	0.24 abc	0.47 ab	5.4 ab	2.5 ab	2.4 ab
2	200	0.26 ab	0.61 ab	4.4 ab	2.8 ab	2.3 ab
2	250	0.25 abc	0.38 ab	4.4 ab	2.9 ab	2.9 ab
3	0	0.24 abc	1.22 a	2.8 ab	2.3 b	2.2 ab
3	100	0.26 abc	1.22 ab	3.3 ab	4.6 a	1.9 ab
3	150	0.23 abc	0.57 ab	3.9 ab	2.5 ab	2.2 ab
3	200	0.22 abc	0.58 ab	2.5 ab	2.6 ab	2.7 ab
3	250	0.23 abc	1.04 ab	3.0 ab	2.5 ab	2.2 ab

Different letters within the columns indicate significant differences between the treatment interactions (Tukey HSD,  $p \leq 0.05$ ). n = 4.

### 3.6. Enzymatic Activity in Tomato Fruits by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Factors

Regarding the potassium iodate factor in the tomato fruits, the  $\text{KIO}_3$  treatments at 150 and 200  $\text{mg L}^{-1}$  significantly increased the GSH content by 9.5 and 14.3%, and the PAL enzymatic activity by 72 and 64%, respectively, in relation to the corresponding control

treatments. GPX, CAT, and APX enzymatic activities were not significantly influenced by  $\text{KIO}_3$  (Figure 3a).



**Figure 3.** Enzymatic antioxidant compounds in tomato fruits: (a) Seed priming based on  $\text{KIO}_3$ ; (b) Seed priming based on  $\text{Na}_2\text{SeO}_3$ . Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

Regarding the sodium selenite factor in the tomato fruits, the GSH content was significantly increased by  $\text{Na}_2\text{SeO}_3$  by 14.3% and 9.5% in the 1 and 2–3  $\text{mg L}^{-1}$  treatments, respectively, in relation to the corresponding control treatments. GPX enzymatic activity significantly increased by 64.3% by  $\text{Na}_2\text{SeO}_3$  in the 3  $\text{mg L}^{-1}$  treatment in relation to the control treatment (Figure S2). PAL, CAT, and APX enzymatic activities were not significantly influenced by  $\text{Na}_2\text{SeO}_3$  in relation to the respective control treatments. APX enzymatic activity significantly increased by 33.3% by  $\text{Na}_2\text{SeO}_3$  in the 2  $\text{mg L}^{-1}$  treatment in relation to the 1  $\text{mg L}^{-1}$  treatment (Figure 3b).

### 3.7. Enzymatic Activity in Tomato Leaves by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Interactions

GSH content and enzymatic activity of GPX in tomato leaves were significantly increased by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions; GSH by 27.3% in the 200–0  $\text{mg L}^{-1}$  interaction and GPX by 80% in the 150–0.5  $\text{mg L}^{-1}$  interaction, in relation to the respective control treatments (0–0  $\text{mg L}^{-1}$  interaction). The enzymatic activities of PAL, CAT, and APX were not significantly modified by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions.

Higher enzymatic activities occurred for PAL in the 0–0.5  $\text{mg L}^{-1}$  interaction, for CAT in the 250–0.5  $\text{mg L}^{-1}$  interaction, and for APX in the 0–2  $\text{mg L}^{-1}$  interaction (Table 5).



**Table 5.** Effect of seed priming based on KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> interactions on the enzymatic activity in tomato leaves.

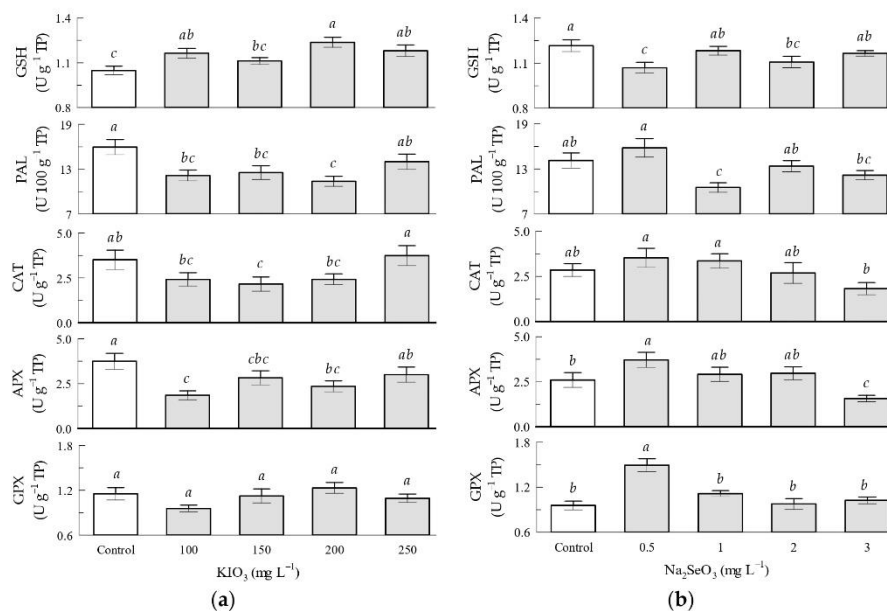
Na <sub>2</sub> SeO <sub>3</sub> (mg L <sup>-1</sup> )	KIO <sub>3</sub> (mg L <sup>-1</sup> )	GSH (U g <sup>-1</sup> TP)	GPX (U g <sup>-1</sup> TP)	PAL (U 100 g <sup>-1</sup> TP)	CAT (U g <sup>-1</sup> TP)	APX (U g <sup>-1</sup> TP)
0	0	1.1 cdefg	1.0 bcd	13.9 abc	3.5 abc	4.6 ab
0	100	1.1 bcdefg	0.9 cd	12.4 abc	2.5 abc	2.1 ab
0	150	1.1 bcdefg	1.0 cd	11.7 abc	1.8 bc	2.4 ab
0	200	1.4 a	0.9 cd	13.8 abc	2.2 abc	1.5 ab
0	250	1.2 abcd	0.7 d	18.6 ab	4.1 abc	2.1 ab
0.5	0	1.0 defg	1.4 abc	19.9 a	2.1 abc	4.2 ab
0.5	100	1.2 abcd	1.1 abcd	17.0 abc	2.5 abc	2.1 ab
0.5	150	1.0 defg	1.8 a	18.7 ab	4.4 abc	4.6 ab
0.5	200	1.0 cdefg	1.7 ab	9.5 c	2.2 abc	3.2 ab
0.5	250	0.9 g	1.2 abcd	13.7 abc	6.3 a	4.2 ab
1	0	1.0 cdefg	1.0 cd	13.9 abc	3.9 abc	2.7 ab
1	100	1.2 abcdef	1.0 cd	9.5 c	3.5 abc	1.2 ab
1	150	1.1 abcdefg	1.1 bcd	8.7 c	1.8 bc	3.1 ab
1	200	1.3 abc	1.1 bcd	8.7 c	2.4 abc	3.1 ab
1	250	1.1 abcdefg	1.2 abcd	11.6 abc	4.9 abc	4.2 ab
2	0	0.9 fg	1.2 abcd	19.1 ab	6.1 ab	4.9 a
2	100	1.0 defg	0.6 d	10.5 bc	2.8 abc	1.6 ab
2	150	0.9 efg	0.7 d	10.9 abc	0.9 c	2.5 ab
2	200	1.1 abcdefg	1.3 abcd	12.5 abc	2.9 bc	2.8 ab
2	250	1.3 ab	1.0 cd	13.7 abc	0.7 c	2.8 ab
3	0	1.1 bcdefg	0.9 cd	12.8 abc	1.8 bc	2.2 ab
3	100	1.1 abcdefg	0.9 cd	11.1 abc	0.6 c	2.1 ab
3	150	1.2 abcde	0.9 cd	12.5 abc	1.7 c	1.2 ab
3	200	1.1 abcdefg	1.0 cd	12.3 abc	2.3 abc	0.8 b
3	250	1.1 abcdefg	1.1 abcd	12.1 abc	2.6 abc	1.4 ab

Different letters within the columns indicate significant differences between the treatment interactions (Tukey HSD,  $p \leq 0.05$ ). n = 4.

### 3.8. Enzymatic Activity in Tomato Leaves by KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> Factors

Regarding the potassium iodate factor in the tomato leaves, by increasing the KIO<sub>3</sub> concentration from 100 to 200 mg L<sup>-1</sup>, the GSH content significantly increased from 10 to 20%, the enzymatic activity of PAL decreased from 23.9 to 28.9%, and the enzymatic activity of APX decreased from 51.4 to 37.8%. The enzymatic activities of CAT and APX were not significantly influenced by KIO<sub>3</sub> (Figure 4a).

Regarding the sodium selenite factor in the tomato leaves, the GSH content significantly decreased by Na<sub>2</sub>SeO<sub>3</sub> by 16.7 and 8.3% in the 0.5 and 2 mg L<sup>-1</sup> treatments, respectively, in relation to the control treatment. The enzymatic activity of GPX significantly increased by 55.6% with Na<sub>2</sub>SeO<sub>3</sub> in the 0.5 mg L<sup>-1</sup> treatment in relation to the control treatment. The enzymatic activities of PAL, CAT, and APX were not significantly influenced by Na<sub>2</sub>SeO<sub>3</sub> (Figure 4b).



**Figure 4.** Enzymatic antioxidant compounds in tomato leaves: (a) Seed priming based on  $\text{KIO}_3$ ; (b) Seed priming based on  $\text{Na}_2\text{SeO}_3$ . Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

### 3.9. Antioxidant Capacity in Tomato Fruits and Leaves by $\text{KIO}_3$ and $\text{Na}_2\text{SeO}_3$ Interactions

Regarding the tomato fruits, the antioxidant capacity of hydrophilic compounds by the ABTS radical significantly decreased by 64.1% in the 200–2  $\text{mg L}^{-1}$  ( $\text{KIO}_3$ - $\text{Na}_2\text{SeO}_3$ ) interaction in relation to the control treatment (0–0  $\text{mg L}^{-1}$  interaction). The antioxidant capacity of lipophilic compounds by ABTS and hydrophilic compounds by DPPH radicals was not significantly influenced by  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions. Higher values of antioxidant capacity of hydrophilic compounds by DPPH occurred in the 0–0.5  $\text{mg L}^{-1}$  interaction (Table 6).

**Table 6.** Effect of seed priming based on  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  interactions on the antioxidant capacity ( $\mu\text{mol TE g}^{-1} \text{DW}$ ) in tomato fruits and leaves.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	Fruits ABTS-H	Fruits ABTS-L	Fruits DPPH-H	Leaves ABTS-H	Leaves ABTS-L	Leaves DPPH-H
0	0	28.7 a	6.6 a	43.7 abc	47.8 ab	20.1 abc	21.5 e
0	100	27.5 ab	8.4 a	34.1 abc	64.7 ab	14.6 bc	22.3 de
0	150	27.0 ab	4.0 a	41.0 abc	44.2 ab	17.7 abc	34.4 bcde
0	200	25.3 ab	3.2 a	42.0 abc	61.6 ab	15.8 abc	26.0 cde
0	250	27.1 ab	7.1 a	44.9 abc	55.3 ab	19.0 abc	36.0 abcde
0.5	0	20.3 abc	8.8 a	50.2 a	47.0 ab	13.1 bc	28.5 cde
0.5	100	19.3 abc	8.4 a	36.6 abc	56.3 ab	10.9 c	36.1 abcde
0.5	150	16.8 abc	4.7 a	41.3 abc	59.4 ab	10.3 c	40.0 abcde
0.5	200	26.4 ab	1.4 a	41.9 abc	45.7 ab	26.2 a	28.9 cde
0.5	250	27.4 ab	3.6 a	36.5 abc	42.7 ab	13.6 bc	22.3 de

Table 6. Cont.

Na <sub>2</sub> SeO <sub>3</sub> (mg L <sup>-1</sup> )	KIO <sub>3</sub> (mg L <sup>-1</sup> )	Fruits ABTS-H	Fruits ABTS-L	Fruits DPPH-H	Leaves ABTS-H	Leaves ABTS-L	Leaves DPPH-H
1	0	17.3 abc	1.8 a	26.2 abc	52.1 ab	18.1 abc	28.0 cde
1	100	16.3 abc	2.3 a	35.6 abc	44.4 ab	12.7 bc	27.0 cde
1	150	27.8 ab	4.3 a	46.9 ab	55.3 ab	19.9 abc	48.3 abcd
1	200	18.1 abc	4.3 a	32.2 abc	44.5 ab	14.0 bc	49.7 abc
1	250	15.3 bc	2.6 a	32.1 abc	46.2 ab	14.0 bc	36.2 abcde
2	0	17.0 abc	1.7 a	29.4 abc	40.4 b	18.9 abc	36.1 abcde
2	100	17.2 abc	4.2 a	23.1 b	44.8 ab	19.7 abc	61.5 a
2	150	12.1 c	6.6 a	20.8 c	44.9 ab	22.5 ab	29.0 cde
2	200	10.3 c	3.4 a	28.3 abc	50.0 ab	18.9 abc	61.7 a
2	250	15.6 abc	2.7 a	26.5 abc	50.0 ab	16.4 abc	52.5 abc
3	0	18.2 abc	9.7 a	28.3 abc	53.0 ab	22.3 ab	56.1 ab
3	100	22.3 abc	9.6 a	30.7 abc	48.0 ab	19.3 abc	49.1 abc
3	150	11.8 c	2.3 a	29.4 abc	67.0 a	18.2 abc	42.7 abcde
3	200	14.9 bc	5.3 a	25.5 abc	54.4 ab	13.4 bc	21.6 e
3	250	23.2 abc	2.4 a	30.9 abc	57.5 ab	20.0 abc	46.9 abcde

-H hydrophilic, -L lipophilic. Different letters within the columns indicate significant differences between the treatment interactions (Tukey HSD,  $p \leq 0.05$ ). n = 4.

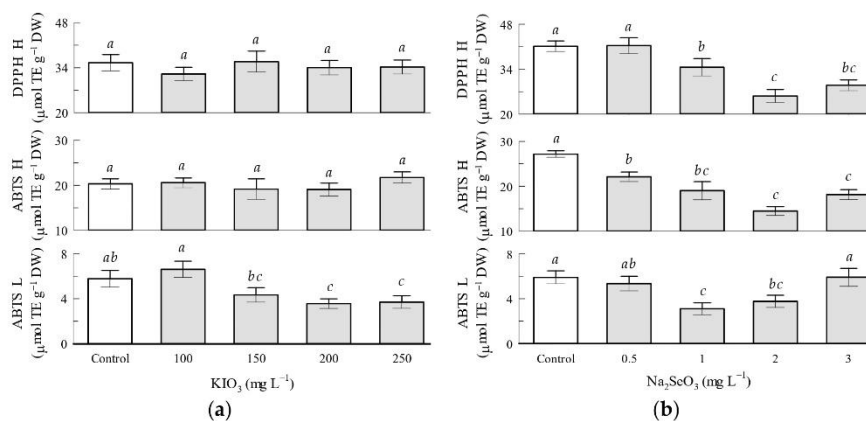
Regarding the tomato leaves, the antioxidant capacity of hydrophilic compounds by DPPH radical significantly increased by 187% in the 200–2 mg L<sup>-1</sup> interaction, in relation to the control treatment. The antioxidant capacity of hydrophilic and lipophilic compounds by the ABTS radicals was not significantly influenced by KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> interactions. Higher values of antioxidant capacity of hydrophilic compounds by ABTS occurred in the 150–3 mg L<sup>-1</sup> interaction and for lipophilic compounds by ABTS in the 200–0.5 mg L<sup>-1</sup> interaction (Table 6).

### 3.10. Antioxidant Capacity in Tomato Fruits and Leaves by KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> Factors

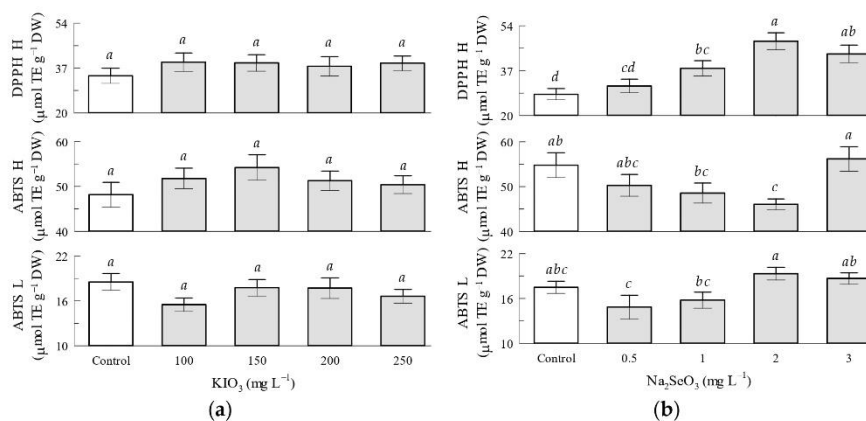
Regarding the potassium iodate factor, the antioxidant capacity of hydrophilic compounds by ABTS and by DPPH and of lipophilic compounds by ABTS was not significantly influenced by KIO<sub>3</sub> in tomato fruits (Figure 5a) and leaves (Figure 6a). The antioxidant capacity of lipophilic compounds by ABTS in tomato fruits increased 88.6% with KIO<sub>3</sub> in the 100 mg L<sup>-1</sup> treatment in relation to the 200 mg L<sup>-1</sup> treatment (Figure 5a).

Regarding the sodium selenite factor in the tomato fruits, the antioxidant capacity of hydrophilic compounds by ABTS and by DPPH was negatively affected by Na<sub>2</sub>SeO<sub>3</sub>, where the 2 mg L<sup>-1</sup> treatment presented higher inhibition of these parameters by 46.9 and 37.7%, respectively (Figure 5b).

Regarding the sodium selenite factor in the tomato leaves, the antioxidant capacity of hydrophilic compounds by DPPH significantly increased by Na<sub>2</sub>SeO<sub>3</sub> 35.4%, 71.8%, and 54.6% in the 1, 2, and 3 mg L<sup>-1</sup> treatments, respectively. The antioxidant capacity of hydrophilic compounds by ABTS significantly decreased by 15.9% by Na<sub>2</sub>SeO<sub>3</sub> in the 2 mg L<sup>-1</sup> treatment in relation to the control treatment. The antioxidant capacity of lipophilic compounds by ABTS was not significantly influenced by Na<sub>2</sub>SeO<sub>3</sub> in tomato fruits and leaves (Figure 6b).



**Figure 5.** Antioxidant capacity of tomato fruits: (a) Seed priming based on  $\text{KIO}_3$ ; (b) Seed priming based on  $\text{Na}_2\text{SeO}_3$ . Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .



**Figure 6.** Antioxidant capacity of tomato leaves: (a) Seed priming based on  $\text{KIO}_3$ ; (b) Seed priming based on  $\text{Na}_2\text{SeO}_3$ . Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

#### 4. Discussion

Implementation of techniques such as seed imbibition is an effective method to improve the response of plants to biotic and abiotic stress conditions (Table S1) through the alteration of antioxidant metabolism [10]. Important findings of seed imbibition are reported, such as the antioxidant response of broccoli influenced by selenium [32], the salt stress tolerance of strawberries influenced by iodine species [33], and the functional effects of selenium in crucifers [34].

Plants can absorb different chemical elements from the soil or the nutrient solution, whether these elements are beneficial or toxic [35]. Selenium can be absorbed through the roots in the form of selenate ( $\text{SeO}_4^{2-}$ ), selenite ( $\text{SeO}_3^{2-}$ ), and organic Se compounds such as selenocysteine (SeCys) and selenomethionine (SeMet) (Table S2), but selenides or



elemental Se cannot be absorbed [36]. The use of selenium in plants has been reported to have positive effects on glutathione content, because selenium increases sulfur (S) receptors and consequently increases the absorption of both elements, favoring the synthesis of secondary metabolites [37].

Plant cells produce free oxygen and its derivatives, such as reactive oxygen species (ROS), which are used as signaling molecules in plants in signal translation in response to environmental conditions; this triggers antioxidant defense mechanisms. In this context, it has been shown that the optimal addition of iodine (Figure S3) and selenium presents an alteration in the ROS production system [38,39], where the enzymatic defense systems, such as catalase, peroxidase, superoxide dismutase, glutathione peroxidase, and ascorbate peroxidase, and non-enzymatic antioxidants, such as glutathione, ascorbate, tocopherols, and phenolic compounds, are activated (Figure S4) to reduce the excessive ROS production [40]. For its part, the SOD enzyme dismutates the  $O_2^-$  into hydrogen peroxide  $H_2O_2$  and molecular oxygen  $O_2$ , and later the catalase (CAT) degrades the  $H_2O_2$  into oxygen and water, while the ascorbate peroxidase uses the ascorbic acid as a donor to stimulate the degradation of  $H_2O_2$ , and the reduced glutathione is responsible for the production of ascorbic acid [41]. Glutathione reductase catalyzes the regeneration of reduced glutathione (GSH) from glutathione disulfide (GSSG) with NADPH as the reducing agent. GSH eliminates  $H_2O_2$  by non-enzymatic reaction with  $O_2^-$  and  $OH^-$ , likewise, GSH has the ability to replenish ascorbic acid through the ascorbate-glutathione cycle, which is of great importance for the antioxidant system [42].

#### 4.1. Non-Enzymatic Compounds

In this research, the use of iodine and selenium concentrations applied to tomato crops by seed priming presented increases in the content of flavonoids, lycopene, and carotene in tomato fruits (Figure S2). These results agree with those reported by Gaucin-Delgado et al. [43], who applied  $2 \text{ mg L}^{-1} \text{ Na}_2\text{SeO}_4$  in the nutrient solution, presenting an increase in the content of phenols in the tomato crop. Likewise, Cunha et al. [44] and Ishtiaq et al. [45] indicated that the use of selenium presents an increase in chlorophyll, carotenes, and phenolic compounds when using concentrations of 7.5 and  $15 \mu\text{g kg}^{-1}$ , while Sabatino et al. [46] indicated that the use of concentrations of 2 and  $4 \mu\text{mol of SeO}_2$  presented a higher content in carotenes in relation to the control. Phenol and vitamin C contents in tomato fruits did not present significant effects between treatments, which are similar to those reported by Smoleń et al. [47], who indicated that the use of  $\text{KIO}_3$  in conjunction with  $\text{Na}_2\text{SeO}_3$  at concentrations of 30 and  $8.5 \mu\text{g dm}^{-3}$ , respectively, did not present a significant effect in relation to the control.

The use of  $\text{KIO}_3$  influenced an increase in the phenol and chlorophyll-*a* contents; however, the  $\text{Na}_2\text{SeO}_3$  treatments did not significantly modify these parameters in the leaves in relation to the control. Similar results were reported by Jerse et al. [48], who mentioned that the use of  $\text{Na}_2\text{SeO}_3$  at  $10 \text{ mg L}^{-1}$  in conjunction with  $\text{KIO}_3$  at concentrations of  $1000 \text{ mg L}^{-1}$  did not present an effect on photosynthetic compounds, likewise indicating that the use of iodine concentrations and selenium separately reduced the dry matter content. However, when both elements interacted, there was a higher biomass content, which is similar to that reported by Smoleń et al. [49], who found that the separate use of selenium and vanadium promotes iodine uptake in plants.

Wang et al. [50] indicated that imbibition treatments present an increase in the content of polyphenols in the rye, which is attributed to the synthesis or activation of a variety of hydrolytic enzymes, causing different alterations in the structure or the synthesis of new compounds with high bioactivity and nutritional value. On the other hand, Vicas et al. [51] indicated that the use of selenium nanoparticles did not affect the phenol content of the broccoli crop. Likewise, Islam et al. [52] indicated that at a higher concentration of  $\text{Na}_2\text{SeO}_3$ , the phenol content begins to decrease. In the same way, Shohag et al. [53] indicated that the imbibed soybean and bean seeds presented a decrease in the phenol content in seeds and sprouts. This decrease in the phenol content is attributed to the imbibition time (Figure S1)

since it is considered that the longer the imbibition time, the greater the water absorption, which presents a dilution effect.

Jerše et al. [48] indicated that the use of iodine and selenium in the imbibition of seeds is a viable method because the enrichment of the pea shoots was achieved with the use of both elements; however, the uptake depends on the shape and/or combinations of the elements. The same effect was reported by Deng et al. [54] and Radawiec et al. [55], who indicated that in osmoconditioning treatments with iron, copper, manganese, zinc, selenium, and iodine in soybean and wheat seeds, they present an increase in the speed of germination and accumulation of these compounds in the shoots, for which they define seed imbibition treatments as a simple and highly efficient technique to increase the content of organic mineral elements in sprouts.

#### 4.2. Enzymatic Activity

Regarding the enzymatic activity, the use of  $\text{KIO}_3$  presented an improvement in the GSH content and a greater enzymatic activity in PAL (Figure S3); similar results are reported by Blasco et al. [56], who indicated that the use of iodide ( $\text{I}^-$ ) and iodate ( $\text{IO}_3^-$ ) in lettuce plants presents an increase in antioxidant enzymes. On the other hand,  $\text{Na}_2\text{SeO}_3$  presented a higher GSH and GPX content (Figure S2); similar results were reported by Zhu et al. [13] and Rady et al. [57], where the use of selenium concentrations favors the increase in the GSH and GPX contents in the tomato crop. The increase in the GSH content is beneficial since high concentrations are needed to overcome oxidative stress in chloroplasts and other organelles [58].

Cao et al. [59], Diao et al. [60], and Kumar et al. [10] indicated that the use of  $\text{Na}_2\text{SeO}_3$  increases the enzymatic activity compared to the control, presenting an increase in GPX, CAT, and APX, while Nawaz et al. [61] indicated that the enzymatic activity of CAT and APX is increased in seed imbibition treatments with Se. Hu et al. [62] indicated that the use of selenium in the seed imbibition solution presents an increase in the  $\alpha$ -amylase content, an increase in the sugar content, and an increase in the enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), in addition to presenting a higher total chlorophyll content and Se content in the seedlings; however, this depends on the imbibition time of the seeds. Nawaz et al. [61] indicated that using selenium and exogenous zinc in seed's imbibition increases germination index, vigor, and enzymatic antioxidants such as catalase, guaiacol peroxidase, superoxide dismutase, and ascorbate peroxidase.

#### 4.3. Antioxidant Capacity

Regarding the antioxidant capacity, there was a positive response when using  $\text{Na}_2\text{SeO}_3$  in the leaves by DPPH of hydrophilic compounds, while Fuentes et al. [63], Medrano Macías et al. [33], and Sarrou et al. [64] indicated that the use of  $\text{KIO}_3$  does not affect the antioxidant capacity in strawberry and tomato crops.

### 5. Conclusions

The application of potassium iodate and sodium selenite in tomato crops by seed imbibition treatments influenced significant changes in non-enzymatic antioxidant compounds, such as phenols, lycopene,  $\beta$ -carotene, and reduced glutathione, as well as on enzymes, that is, phenylalanine ammonium lyase, catalase, and ascorbate peroxidase, both in tomato leaves and fruits; however, the same treatments influenced not significant changes in the antioxidant capacity. Seed priming based on trace elements is a useful and simple technique to perform in agricultural and horticultural production systems. Although this method does not present inconvenience due to the low concentrations of trace elements required, it is necessary to carry out more studies to establish the optimal concentrations according to the crop and the form of application, which allow for improvement of the desired indicators, such as the antioxidant compound pool of the edible organs of the plants, understand the balance and pathway of trace elements in the plant, and the benefits of biofortification of the fruits.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antiox12061265/s1>. Table S1: List of some patented seed priming treatments commercially available; Table S2: Summary of selenoprotein functions; Figure S1: Ranges of selenium application in several crops, applications form to achieve benefits such as biofortification and stimulation; Figure S2: Biochemical and ionic effects of selenium and nanoselenium application in plants; Figure S3: Uptake, transport and metabolism of iodine in plants; Figure S4: Iodine as a modulator of antioxidants.

**Author Contributions:** Conceptualization, Á.M.-M. and F.M.-R.; methodology, F.M.-R.; formal analysis, A.B.-M.; investigation, F.M.-R.; resources, Á.M.-M., A.B.-M. and S.G.-M.; data curation, F.M.-R. and Á.M.-M.; writing—original draft preparation, F.M.-R.; writing—review and editing, Á.M.-M. and A.B.-M.; visualization, Á.M.-M., A.J.-M., A.B.M.-D. and F.M.L.-V.; supervision, S.G.-M. and A.B.-M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Data shall be available through request to the corresponding author.

**Acknowledgments:** Fernando Mejía-Ramírez gives thanks for the financial support sponsored by the National Council of Humanities, Science and Technology of Mexico (CONAHCYT) for doctoral studies.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## SEGUNDO ARTICULO

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

1 **Effect of root imbibition with Selenium and Iodine on**  
 2 **antioxidant compounds in tomato (*Solanum***  
 3 ***lycopersicum* L.) crop**  
 4

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20  
 21 **Abstract**  
 22

23 The use of trace elements such as iodine and selenium in agriculture is gaining great importance  
 24 due to the benefits in plants before different types of biotic or abiotic stress. This research aimed to  
 25 evaluate the seedling root priming with Na<sub>2</sub>SeO<sub>3</sub> (0, 0.5, 1, 2, 3 mg L<sup>-1</sup>) and KIO<sub>3</sub> (0, 100, 150, 200,  
 26 250 mg L<sup>-1</sup>) on the antioxidant compounds of tomato (*Solanum lycopersicum* L.) fruits and leaves. The  
 27 crop was established under greenhouse conditions in 10-L polyethylene containers containing peat  
 28 moss and perlite 1:1 (v/v), in a randomized complete block experimental design with a 5<sup>2</sup> factorial  
 29 arrangement. In the fruits, the Na<sub>2</sub>SeO<sub>3</sub> influenced the GHS, flavonoids, lycopene and β-carotene  
 30 contents, while the KIO<sub>3</sub> influenced the GHS, vitamin C and lycopene contents. The KIO<sub>3</sub>-Na<sub>2</sub>SeO<sub>3</sub>  
 31 interactions affected the GSH, phenols, flavonoids, lycopene and β-carotene contents in fruits. In the  
 32 leaves the GHS content increased with the Na<sub>2</sub>SeO<sub>3</sub>, while the GSH, flavonoids, and chlorophyll  
 33 contents increased with the KIO<sub>3</sub> factor and KIO<sub>3</sub>-Na<sub>2</sub>SeO<sub>3</sub> interactions. The evaluated enzymes in  
 34 fruits and leaves decreased with the both the KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> concentrations. The Na<sub>2</sub>SeO<sub>3</sub>  
 35 influenced the hydrophilic compounds by ABTS and DPPH, while the KIO<sub>3</sub> influenced the  
 36 hydrophilic compounds by ABTS. In the leaves, the KIO<sub>3</sub> influenced the lipophilic compounds by  
 37 ABTS. The KIO<sub>3</sub>-Na<sub>2</sub>SeO<sub>3</sub> interactions influenced the hydrophilic compounds by ABTS in both the  
 38 fruits and leaves. Seedling root imbibition in KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>3</sub> is a method that implemented in the  
 39 tomato crop presents interesting aspects in the increase of the antioxidant capacity and the non-  
 40 enzymatic compounds, such as vitamin C, phenols, flavonoids and GSH contents. However, this  
 41 method presented an inhibition in the antioxidant enzymes.  
 42

43 **Keywords:** Antioxidant, KIO<sub>3</sub>, Na<sub>2</sub>SeO<sub>3</sub>, ROS, secondary metabolites.  
 44

45 **Introduction**  
 46

47 The use of trace elements such as iodine (I) and selenium (Se) in agriculture is a practice that is  
 48 gaining great importance and relevance worldwide, because I and Se can promote growth and the  
 49 potential for tolerance to plant stress when applied in low concentrations (Hasanuzzaman *et al.*, 2010).  
 50 Selenium (Se) is classified as an inorganic plant biostimulant, it has shown an improvement in the  
 51 absorption of nutrients, increases the tolerance of plants to stress and improves the quality of crop

2

52 yields (de Mello Prado, 2021; Dima *et al.*, 2020; Hasanuzzaman *et al.*, 2020). I and Se in low  
53 concentrations can function as signalers to improve the plant's defense system, which is reflected in  
54 the increase in the content of secondary metabolites; however, in high concentrations it can cause  
55 oxidative damage to tissues (Abedi *et al.*, 2021; Mittler, 2017).

56 Plants have the ability to absorb chemical elements from the soil and from the nutrient solution,  
57 whether these are nutrients or non-nutrients, as well as beneficial or toxic (Kathpalia and Bhatla,  
58 2018). Se is absorbed by plant roots in the rhizosphere solution in its organic form as selenocysteine  
59 (SeCys) and selenomethionine (SeMet), and its inorganic form as selenate ( $\text{SeO}_4^{2-}$ ) and selenite  
60 ( $\text{SeO}_3^{2-}$ ), but selenides or elemental Se cannot be absorbed by plant roots (White, 2018).

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

67 Iodine can be absorbed from the soil through the plant roots as organic iodine ions, iodate ( $\text{IO}_3^-$ )  
68 and iodide ( $\text{I}^-$ ), and from the atmosphere in gaseous form by the plant leaves as molecular iodine ( $\text{I}_2$ )  
69 and methyl iodide ( $\text{CH}_3\text{I}$ ) (Medrano-Macías *et al.*, 2016).

70 Iodine and selenium play important roles with benefit in crop plants particularly under stress  
71 conditions, presenting positive effects in reducing the  $\text{H}_2\text{O}_2$  and  $\text{O}_2^-$  contents (Zhu *et al.*, 2017), that is,  
72 have an effect on the activation of the defense system to control the production and accumulation of  
73 reactive oxygen species (ROS). In this context, the plant cell system increases the levels of non-  
74 enzymatic antioxidant metabolites, including glutathione, ascorbate, tocopherol, phenolic compounds,  
75 anthocyanins (Halka *et al.*, 2019; Huang *et al.*, 2019), and a wide network of enzymatic antioxidants,  
76 such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX) and glutathione  
77 reductases (GR), among others (Mittler *et al.*, 2004; Revelou *et al.*, 2022).

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

81

## 82 **Materials and Methods**

83

### 84 *Crop establishment*

85 Tomato crop was established in a tunnel-type greenhouse with plastic cover and natural  
86 ventilation in the Horticulture Department at the Universidad Autónoma Agraria Antonio Narro, in  
87 Saltillo, Mexico (25° 21' NL, 101° 01' WL, altitude 1743 m). The average conditions in the  
88 greenhouse in the crop cycle were: temperature 21 °C, relative humidity 51%, solar radiation 735 W  
89  $\text{m}^{-2}$ , photosynthetically active radiation 568  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

90

### 91 *Preparation of treatments*

92 Sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) (99%, Sigma Aldrich, St. Louis, MO, USA) and Potassium iodate  
93 ( $\text{KIO}_3$ ) (99%, Sigma Aldrich, St. Louis, MO, USA) and were used. A sodium selenite stock solution  
94 (1000 ppm) was prepared. A mass of 21.89 mg of  $\text{Na}_2\text{SeO}_3$  was gauged to 10 mL with distilled water.  
95 Dilutions of 0.5, 1, 2 and 3 mL of the stock solution were gauged to 1 L with distilled water to obtain  
96 the treatments of 0.5, 1, 2, and 3  $\text{mg L}^{-1}$ , respectively. Also a potassium iodate stock solution (1000  
97 ppm) was prepared. A mass of 1.68 g of  $\text{KIO}_3$  was gauged to 1 L with distilled water, and the dilutions  
98 of 100, 150, 200 and 250 mL of the stock solution were gauged to 1 L with distilled water to obtain  
99 the treatments of 100, 150, 200, and 250  $\text{mg L}^{-1}$ , respectively. Control treatments consisted of  
100 imbibition in distilled water (Table 1).

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

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )
--	---------------------------------------

4

0	0
0.5	100
1	150
2	200
3	250

102 25 treatments ( $5^2$  factorial), n = 4 replications, 100 experimental units.

103

104

#### *Sowing and root priming*

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#### *Planting and crop management*

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#### *Sampling*

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### ***Non-enzymatic compounds***

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#### *Vitamin C*

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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<sup>-1</sup> FW).

$$\text{Vitamin C} = \frac{\text{mL of 2,6 dichlorophenolindophenol} \times 0.088 \times \text{total volume} \times 100}{\text{aliquot volume} \times \text{sample weight}} \quad (1)$$

134

135

#### *Phenols*

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#### *Flavonoids*

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144

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<sup>-1</sup> DW).

#### *Chlorophyll*

6

145 Chlorophyll content was quantified using the method proposed by Munira *et al.* (2015), by  
 146 reading the absorbances at 663 and 645 nm wavelengths, and the results were computed (Equations 2,  
 147 3 and 4) and expressed in  $\mu\text{g g}^{-1}$  FW).

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

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

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

148

149 *Lycopene and  $\beta$ -carotene*

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

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

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

153

154 *Extraction*

155 Samples of leaves and rape fruits of tomatoes were freeze-dried and macerated by using a  
 156 mortar and pestle; 200 mg of dry tissue and 20 mg of polyvinyl pyrrolidone were added in a 2-mL  
 157 centrifuge tube; 1.5 mL of phosphate buffer (0.1 M, pH 7-7.2) was added; and the mixture was  
 158 subjected to sonication for 5 min, and then centrifuged in a Prism C2500 refrigerated microcentrifuge  
 159 (Labnet International Inc., Edison, NJ, USA) at 12,500 rpm for 10 min at 4 °C. The supernatant was  
 160 collected and filtered with a 0.45-mm-diameter nylon membrane. Finally, the supernatant was diluted  
 161 (1:20) with phosphate buffer (0.1 M, pH 7-7.2). This dilution was used to analyze the absorbances of  
 162 reduced glutathione (GSH), glutathione peroxidase (GPX), phenylalanine ammonium lyase (PAL),  
 163 catalase (CAT), and ascorbate peroxidase (APX) in a GENESYS 10S UV-Vis Spectrum (Thermo  
 164 Fisher Scientific, Inc., Waltham, MA, USA), as well as the antioxidant capacity of ABTS and DPPH  
 165 radicals in a BK-EL10C Elisa microplate reader (BIOBASE, Jinan, Shandong, China) at the  
 166 corresponding wavelengths.

167

168 *Reduced Glutathione (GSH)*

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

172

173 ***Enzymatic Activity***

174

175 *Glutathione Peroxidase (GPX) (QE 1.11.1.9)*

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

179

180

181

182

183 *Phenylalanine Ammonium Lyase (PAL) (QE 4.3.1.5)*

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

187

188 *Catalase (CAT) (QE 1.11.1.6)*



8

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

192

193 *Ascorbate Peroxidase (APX) (EC 1.11.1.11)*

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

197

### 198 **Antioxidant Capacity**

199

200 *Hydrophilic and Lipophilic Compounds by ABTS*

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

205

206 *Hydrophilic Compounds by DPPH*

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

210

211 *Statistical Analyses*

212 The results were analyzed by analysis of variance to determine the variables that presented a  
 213 significant statistical difference ( $p \leq 0.05$ ) so that the variables with significant effects were submitted  
 214 to comparison means tests by Tukey ( $p \leq 0.05$ ) using the statistical software InfoStat® 2020e.

215

### 216 **Results and Discussion**

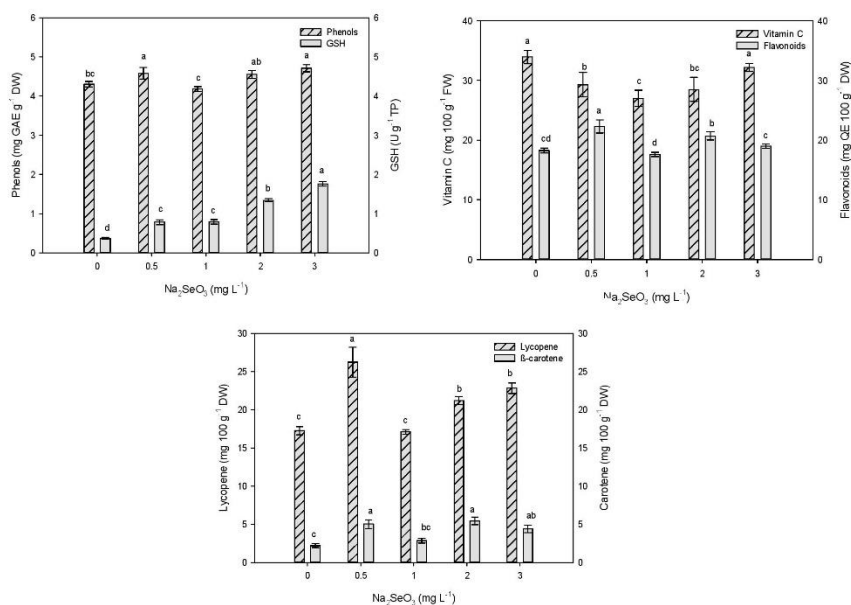
217

218 *Non-enzymatic compounds in tomato fruits by  $Na_2SeO_3$*

219 Regarding the sodium selenite factor in the tomato fruits, the GSH content significantly  
 220 increased 5.6 times in the dose of  $3\ mg\ L^{-1}$ , while the vitamin C content significantly decreased in the  
 221 doses of  $0.5, 1$  and  $2\ mg\ L^{-1}$ , in relation to the control treatments. On the other hand, the total phenols  
 222 content significantly increased by 4 and 9% in the doses of  $0.5$  and  $3\ mg\ L^{-1}$  in relation to the control  
 223 treatment, similar results were reported by Abedi *et al.* (2021) and Andrejiová *et al.* (2016), who  
 224 indicated that the use of selenium favors the increase of polyphenols in the tomato crop. The  
 225 flavonoid, lycopene and  $\beta$ -carotene contents significantly increased by 22, 52 and 127%, respectively,  
 226 in the dose of  $0.5\ mg\ L^{-1}$  in relation to the control treatments (Figure 1), similar results were reported  
 227 by Rady *et al.* (2020), who indicated that the use of selenium favors the increase in the lycopene  
 228 content in tomato fruits, while Gaucin-Delgado *et al.* (2020) and Sabatino *et al.* (2021) indicated that  
 229 the use of selenium presents important aspects in the nutraceutical quality in tomato fruits, presenting  
 230 increases in the carotenoid, polyphenol, vitamin C and lycopene contents.

231

10



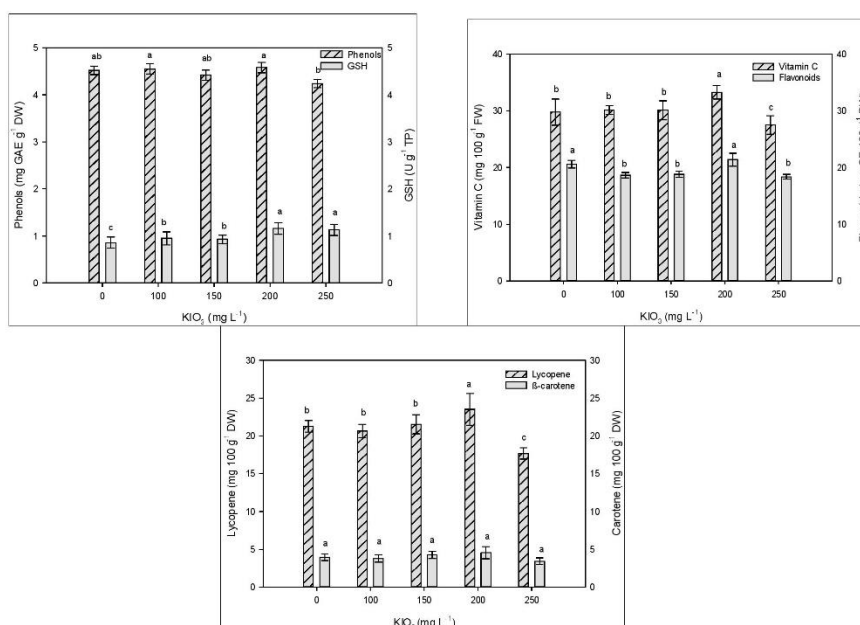
232 **Figure 1.** Effect of root imbibition by Na<sub>2</sub>SeO<sub>3</sub> in the non-enzymatic antioxidant compounds in  
 233 tomato fruits. Different letters indicate significant differences between treatments (Tukey HSD,  $p$   
 234  $\leq 0.05$ ).  $n = 4$ .  
 235

236 *Non-enzymatic compounds in tomato fruits by KIO<sub>3</sub>*

237 The root imbibition with potassium iodate significantly influenced the contents of non-  
 238 enzymatic compounds in the tomato fruits. The GSH and vitamin C contents increased by 37 and  
 239 11.7%, respectively, in the dose of 200 mg L<sup>-1</sup>, in relation to the corresponding control treatments. Li  
 240 *et al.* (2017a) reported that the use of iodine concentrations favors the increase in vitamin C content in  
 241 chili crop, which tends to decrease at high iodine concentrations. This stimulation effect of iodine at  
 242 low concentrations to increase the vitamin C content also was observed in strawberry crop by Li *et al.*  
 243 (2017b) with applications either as iodide or iodate in the nutrient solution in concentrations lower  
 244 than 1 mg L<sup>-1</sup>, while higher iodine concentrations influenced the decrease the ascorbic acid content in  
 245 relation to the control treatment. The phenols, flavonoids and lycopene contents increased in the dose  
 246 of 200 mg L<sup>-1</sup>, while the  $\beta$ -carotene content was not significantly affected in relation to the control  
 247 treatments (Figure 2). Opposite results were reported by Smoleń *et al.* (2015), where the use of KI and  
 248 KIO<sub>3</sub> did not significantly influence the carotenoids, flavonoids and phenols contents in relation to the  
 249 control treatments.  
 250



12



251  
252 **Figure 2.** Effect of root imbibition by  $KIO_3$  in the non-enzymatic antioxidant compounds in  
253 tomato fruits. Different letters indicate significant differences between treatments (Tukey HSD,  $p$   
254  $\leq 0.05$ ).  $n = 4$ .  
255

#### 256 *Non-enzymatic compounds in tomato fruits by $Na_2SeO_3$ and $KIO_3$ interactions*

257 The GSH content in the tomato fruits significantly increased 9 times in the 3-100  $mg L^{-1}$   
258 interaction, in relation to the control treatment (0-0  $mg L^{-1}$  interaction). The vitamin C content tended  
259 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  
260 that  $KIO_3$  have a negative effect in the ascorbic acid content when it is combined with  $Na_2SeO_3$  at 2  
261  $mg L^{-1}$ . The 0.5-200  $mg L^{-1}$  interaction significantly influenced the increase of phenols, flavonoids,  
262 lycopene and  $\beta$ -carotenes contents in 1.23, 1.66, 2.13 and 2.76 times, respectively, in relation to the  
263 control treatments (Table 2), for which this interaction presents important aspects in nutraceutical  
264 quality and postharvest quality, because the flavonoid contributes to delay the ripening of tomato fruits  
265 (Zhang *et al.*, 2015).  
266

267 **Table 2.** Effect of root imbibition by  $Na_2SeO_3$  and  $KIO_3$  interactions in the non-enzymatic  
268 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 ( $mg QE 100 g^{-1} DW$ )	Lycopene ( $mg 100 g^{-1} DW$ )	$\beta$ -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	17.4ghij	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

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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	17.5ghi	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	4.6abcd	17.7efghi	23.2cdef	4.9ab
3	200	2.0a	31.1cdef	4.3bcde	17.5ghi	25.2bcd	4.2ab
3	250	1.9a	34.7abcd	4.6abcd	17.9defghi	18.6fghijk	4.1ab

269 Different letters within the columns indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

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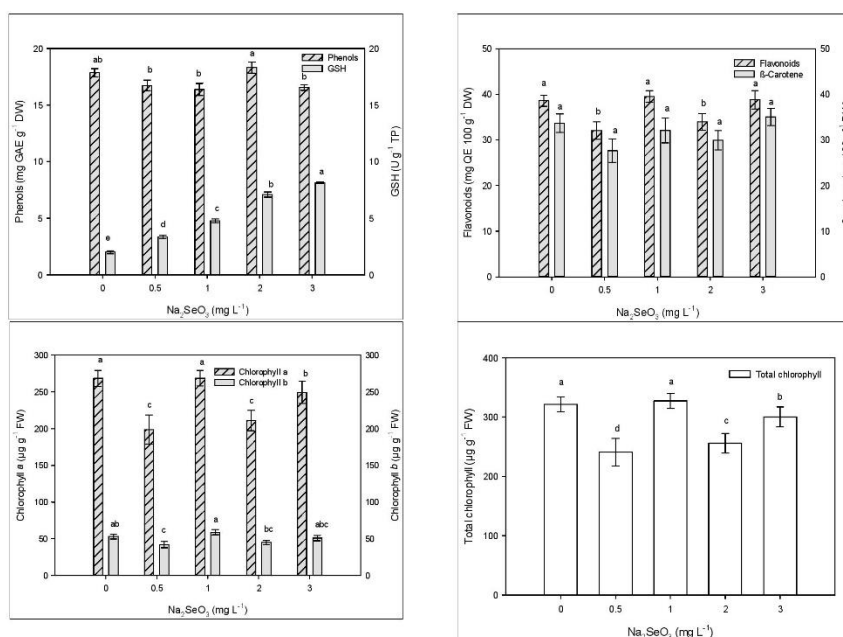
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#### Enzymatic compounds in tomato leaves by $\text{Na}_2\text{SeO}_3$

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  $3 \text{ mg L}^{-1}$  in relation to the control treatment. Rady *et al.* (2020) found that the use of selenium influence 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  $3 \text{ mg L}^{-1}$  in relation to the control treatment. The flavonoid content were not significantly modified by  $\text{Na}_2\text{SeO}_3$  (Figure 3), Zhang *et al.* (2023) also reported not significant results in the flavonoid content with  $\text{Na}_2\text{SeO}_3$  applied by foliar spraying and through irrigation. The chlorophyll contents significantly decreased in the dose of  $0.5 \text{ mg L}^{-1}$  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  $\beta$ -carotene contents as the  $\text{Na}_2\text{SeO}_3$  concentration was higher; however, Alsamadany *et al.* (2023) indicated that the use of selenium favored the increase in the chlorophyll content, while the  $\beta$ -carotene content were not significantly modified, in relation to the control treatments. Cunha *et al.* (2022) and Ishtiaq *et al.* (2023) indicated that the use of selenium influence the increase of chlorophyll,  $\beta$ -carotene and phenolic compounds with concentrations from  $7.5$  to  $15 \mu\text{g kg}^{-1}$ , 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).

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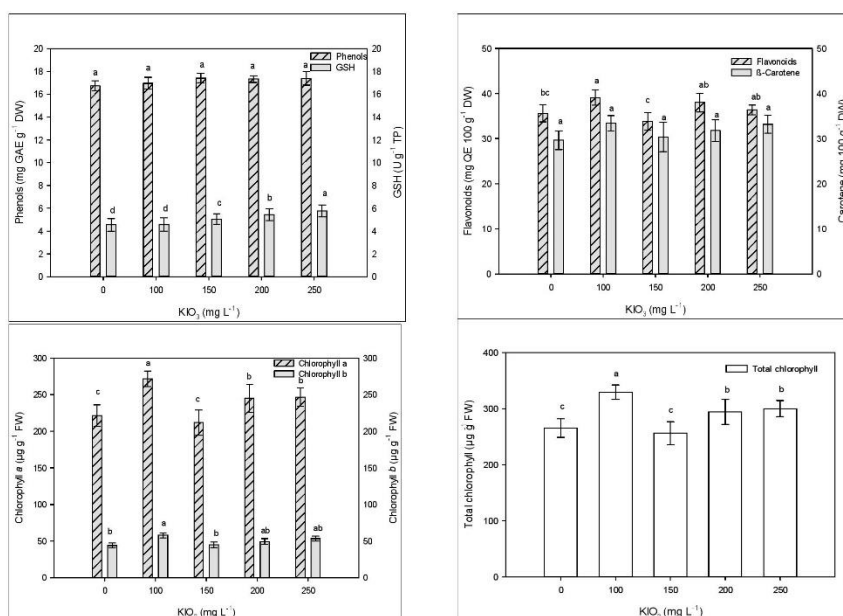


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295 **Figure 3.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  in the non-enzymatic antioxidant compounds in  
296 tomato leaves. Different letters indicate significant differences between treatments (Tukey HSD,  $p$   
297  $\leq 0.05$ ).  $n = 4$ .

#### 298 *Non-enzymatic compounds in tomato leaves by KIO<sub>3</sub>*

299 In the tomato leaves the potassium iodate significantly influenced an increase of 26% of the  
300 GSH content in the dose of  $250 \text{ mg L}^{-1}$ , in relation to the control treatment. The phenol content was  
301 not significantly modified by  $\text{KIO}_3$ , similar results are those reported by Puccinelli *et al.* (2021) who  
302 indicated that the use of KI in the lettuce crop did not a significant influence the phenol content. The  
303 flavonoid content significantly increased by 9.8% in the dose of  $100 \text{ mg L}^{-1}$  of  $\text{KIO}_3$  in relation to the  
304 control treatment. The greater chlorophyll contents were presented in the dose of  $100 \text{ mg L}^{-1}$  of  $\text{KIO}_3$   
305 in relation to the control treatments, these results agree with those reported by Li *et al.* (2017a) who  
306 indicated that the use of iodine improves the chlorophyll content, however, as the iodine concentration  
307 increases, the chlorophyll content begins to decrease. Regarding the  $\beta$ -carotene content, there was no  
308 significant difference between the treatments (Figure 4). Krzepilko *et al.* (2023) indicated that the use  
309 of iodine influences the increase in the chlorophyll content, however, in the carotenoid content, the  
310 applications either as KI or  $\text{KIO}_3$  do not influence the carotenoid content.  
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313 **Figure 4.** Effect of root imbibition by  $\text{KIO}_3$  in the non-enzymatic antioxidant compounds in  
314 tomato leaves. Different letters indicate significant differences between treatments (Tukey HSD,  $p$   
315  $\leq 0.05$ ).  $n = 4$ .  
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317 *Non-enzymatic compounds in tomato leaves by  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interactions*

318 The higher significant GSH content in the tomato leaves reached 7.25 times in the 2-250  $\text{mg L}^{-1}$   
319 ( $\text{Na}_2\text{SeO}_3$  -  $\text{KIO}_3$ ) interaction in relation to the control treatment (0-0  $\text{mg L}^{-1}$  interaction), which  
320 evidences the importance of the glutathione role in the control of reactive oxygen species (ROS) that  
321 accumulate during biotic stress, and the GSH reduces the cell damage (Gullner *et al.*, 2017;  
322 Zechmann, 2020). The phenol and  $\beta$ -carotene contents in the tomato leaves did not show significant  
323 effects. Regarding the flavonoid content, the tomato leaves reached an increase of 41.2% in the 0.5-  
324 100  $\text{mg L}^{-1}$  interaction, and 51.8% in the 3-200  $\text{mg L}^{-1}$  interaction, in relation to the control treatment,  
325 however the 10.5% surplus implies an increase in the cost by  $6(\text{Na}_2\text{SeO}_3) + 2(\text{KIO}_3)$ . Golubkina *et al.*  
326 (2018) reported that the use of iodine and selenium together influence favorable effects by increasing  
327 the content of flavonoids, while Smoleń *et al.* (2019) indicated that the application of iodine and  
328 selenium together influence the increase of plant metabolism, which is reflected in the increase of  
329 antioxidant compounds in the face of the stress by which the plants are found. The chlorophyll-*a*,  
330 chlorophyll-*b* and total chlorophyll contents significantly increased by 34, 80.6 and 40.9%,  
331 respectively, in the 1-100  $\text{mg L}^{-1}$  interaction, compared to the control treatments (Table 3).  
332

333 **Table 3.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interactions in the non-enzymatic  
antioxidant compounds in tomato leaves.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	GSH ( $\text{U g}^{-1}$ TP)	Phenols ( $\text{mg AG g}^{-1}$ DW)	Flavonoids ( $\text{mg QE } 100 \text{ g}^{-1}$ DW)	Chl- <i>a</i> ( $\mu\text{g g}^{-1}$ )	Chl- <i>b</i> ( $\mu\text{g g}^{-1}$ )	Total Chl. ( $\mu\text{g g}^{-1}$ )	$\beta$ carotene ( $\text{mg } 100 \text{ g}^{-1}$ 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

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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	19.5 <sup>a</sup>	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,  $p \leq 0.05$ ).  $n = 4$ .

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#### Enzymatic compounds of tomato fruits by $\text{Na}_2\text{SeO}_3$

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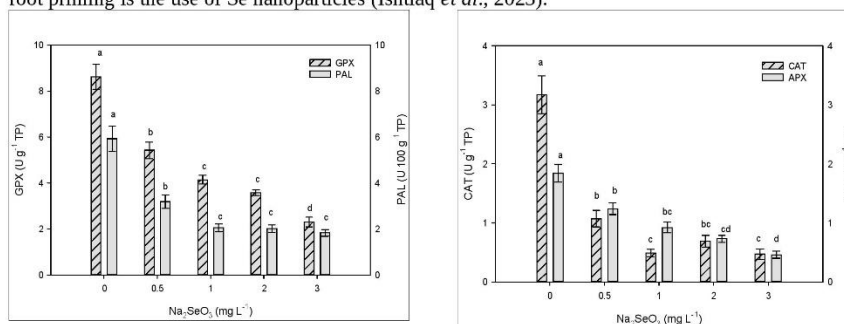
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The sodium selenite applied by imbibition to the seedling roots negatively influenced the enzymatic activity in the tomato fruits. The GPX, PAL, CAT y APX enzymatic activities significantly decreased by 73.1, 68.1, 85.3 and 73.9%, respectively, in the dose of  $3 \text{ mg L}^{-1}$  in relation of the control treatments (Figure 5), which indicates that  $\text{Na}_2\text{SeO}_3$  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 (Bermi *et al.*, 2019; Sali *et al.*, 2018). An alternative for improving the enzymatic activity in tomato cultivation from the root priming is the use of Se nanoparticles (Ishtiaq *et al.*, 2023).



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**Figure 5.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  in the enzymatic compounds in tomato fruits.

Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

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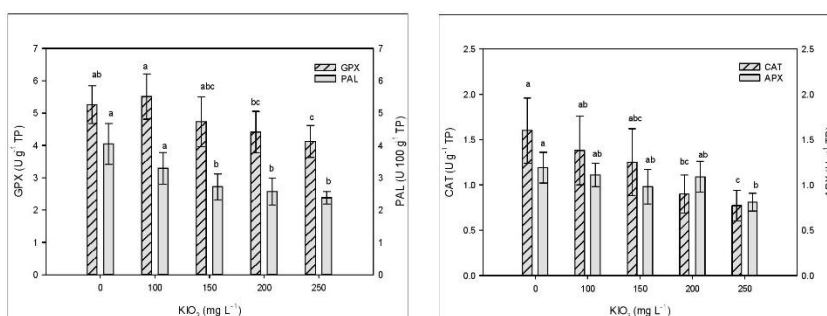
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#### Enzymatic compounds of tomato fruits by $\text{KIO}_3$

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  $250 \text{ mg L}^{-1}$  in relation of the control treatments (Figure 6). The CAT and APX enzymes are responsible for degrading  $\text{H}_2\text{O}_2$  in water, such process was inhibited due to enzymatic activity was decreased as the  $\text{KIO}_3$  dose increased.

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**Figure 6.** Effect of root imbibition by  $\text{KIO}_3$  in the enzymatic compounds in tomato fruits. Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

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*Enzymatic compounds of tomato fruits by  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interactions*

The enzymatic activity in the tomato fruits significantly decreased as the doses of treatments of  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interaction increased (Table 4). The enzymes in the plant reduce the level of reactive oxygen species (ROS), where catalase degrades  $\text{H}_2\text{O}_2$  into oxygen and water, while ascorbate peroxidase uses ascorbic acid as a donor to stimulate the  $\text{H}_2\text{O}_2$  degradation, while reduced glutathione is responsible for the production of ascorbic acid (Hussain *et al.*, 2019; Zhu *et al.*, 2017).

**Table 4.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interactions in the enzymatic compounds in tomato fruits.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	PAL ( $\text{U } 100 \text{ g}^{-1} \text{ TP}$ )	GPX ( $\text{U g}^{-1} \text{ TP}$ )	CAT ( $\text{U g}^{-1} \text{ TP}$ )	APX ( $\text{U g}^{-1} \text{ TP}$ )
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,  $p \leq 0.05$ ).  $n = 4$ .

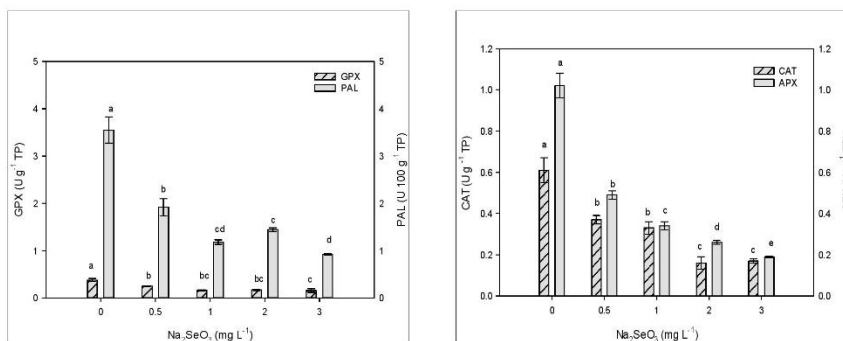
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*Enzymatic compounds of tomato leaves by  $\text{Na}_2\text{SeO}_3$*

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  $3 \text{ mg L}^{-1}$  in relation of the control treatments (Figure 7). Rady *et al.* (2020) indicated that the use of  $\text{Na}_2\text{SeO}_3$  at 25

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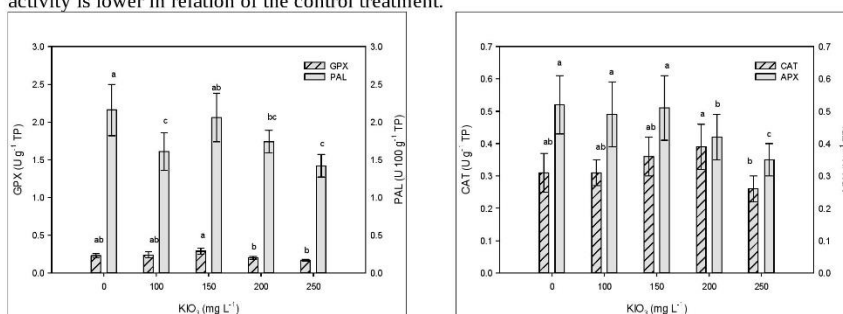
377 and 50 mM applied by foliar spraying influence the increase of enzymatic activity, while Cunha  
 378 *et al.* (2022) reported an increase in the CAT and APX enzymatic activities in the lowest  
 379 concentration ( $7.5 \mu\text{g kg}^{-1}$ ), and as the concentration increased the enzyme activities decreased.  
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 382 **Figure 7.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  in the enzymatic compounds in tomato leaves.  
 383 Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .  
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#### 385 *Enzymatic compounds of tomato leaves by KIO<sub>3</sub>*

386 The potassium iodate applied by imbibition to the seedling roots negatively influenced the  
 387 enzymatic activity in the tomato leaves. The GPX, PAL, CAT y APX enzymatic activities  
 388 significantly decreased by 2.3, 34.1, 11.5 and 31.7%, respectively, in the dose of  $250 \text{ mg L}^{-1}$   
 389 in relation of the control treatments (Figure 8), which reflects that in higher concentrations the  
 390 iodine presents an oxidative stress, due to the CAT and APX are enzymatic antioxidants can  
 391 catalyze the decomposition of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$ , which protect the cells from excess  $\text{H}_2\text{O}_2$ .  
 392 Li *et al.* (2017a) reported that as higher the iodine concentration in seedlings, the enzymatic  
 393 activity is lower in relation of the control treatment.



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 395 **Figure 8.** Effect of root imbibition by  $\text{KIO}_3$  in the enzymatic compounds in tomato leaves.  
 396 Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .  
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#### 398 *Enzymatic compounds of tomato leaves by Na<sub>2</sub>SeO<sub>3</sub> and KIO<sub>3</sub> interactions*

399 The enzymatic activity in the tomato leaves significantly decreased as the concentrations of the  
 400 treatments of the  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  Interactions were increased (Table 5).  
 401

402 **Table 5.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  and  $\text{KIO}_3$  interactions in the enzymatic  
 403 compounds in tomato leaves.

$\text{Na}_2\text{SeO}_3$ ( $\text{mg L}^{-1}$ )	$\text{KIO}_3$ ( $\text{mg L}^{-1}$ )	PAL ( $\text{U } 100 \text{ g}^{-1} \text{ TP}$ )	GPX ( $\text{U g}^{-1} \text{ TP}$ )	CAT ( $\text{U g}^{-1} \text{ TP}$ )	APX ( $\text{U g}^{-1} \text{ TP}$ )
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0	0	4.6 <sup>a</sup>	0.4abc	0.59abc	1.17 <sup>a</sup>
0	100	3.7ab	0.5 <sup>a</sup>	0.5abcd	1.17 <sup>a</sup>
0	150	4.3 <sup>a</sup>	0.4ab	0.68ab	1.17 <sup>a</sup>
0	200	2.4cde	0.3abcd	0.84 <sup>a</sup>	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

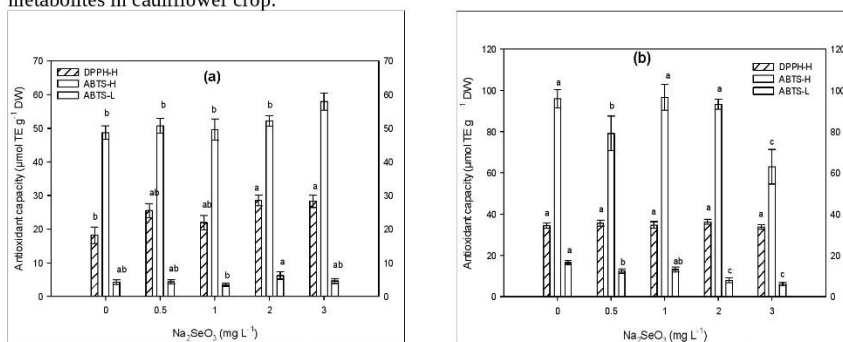
Different letters within the columns indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

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#### Antioxidant capacity of tomato fruits and leaves by $\text{Na}_2\text{SeO}_3$

406 The sodium selenite positively influenced the antioxidant capacity in tomato fruits. The  
407 hydrophilic compounds by ABTS and by DPPH radicals increased by 55.6 and 18.4%,  
408 respectively, in the dose of  $3 \text{ mg L}^{-1}$  in relation to the control treatments (Figure 9a). The  
409 lipophilic compounds by ABTS radical in tomato fruits were not significantly influenced by  
410  $\text{Na}_2\text{SeO}_3$ .

411 The sodium selenite negatively influenced the antioxidant capacity in tomato leaves. The  
412 hydrophilic and lipophilic compounds by ABTS radical decreased by 35.1 and 66.7%,  
413 respectively, in the dose of  $3 \text{ mg L}^{-1}$  in relation to the control treatments (Figure 9b). The  
414 hydrophilic compounds by DPPH radical in tomato leaves were not significantly influenced by  
415  $\text{Na}_2\text{SeO}_3$ . Saeedi *et al.* (2021) reported that the exogenous application of selenium presented  
416 favorable aspects in the antioxidant activity, as well as the improvement of secondary  
417 metabolites in cauliflower crop.  
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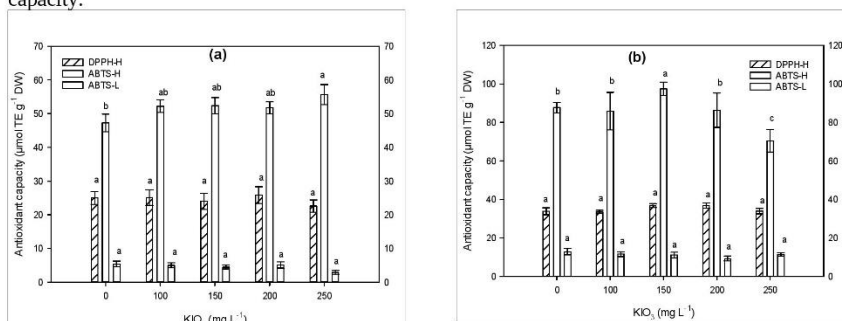
**Figure 9.** Effect of root imbibition by  $\text{Na}_2\text{SeO}_3$  in the antioxidant capacity of tomato fruits and leaves. Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

#### Antioxidant capacity of tomato fruits and leaves by $\text{KIO}_3$



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425 The antioxidant capacity of hydrophilic compounds by ABTS radical influenced by potassium  
 426 iodate, significantly increased in the tomato fruits by 17.7% (Figure 10a) and significantly  
 427 decreased in the tomato leaves by 20.5% (Figure 10b) both the two in the dose of 250 mg L<sup>-1</sup> in  
 428 relation to the control treatments. The lipophilic compounds by ABTS radical and the  
 429 hydrophilic compounds by DPPH radical in tomato fruits and leaves were not significantly  
 430 influenced by KIO<sub>3</sub>. Medrano Macías *et al.* (2021) obtained statistical difference in the  
 431 antioxidant capacity of strawberry with KIO<sub>3</sub> by the hydrophilic ABTS method, while the  
 432 lipophilic ABTS and DPPH methods there were no effected. Sarrou *et al.* (2019) and Smoleń  
 433 *et al.* (2015) reported that the use of KI and KIO<sub>3</sub> does not present an effect on the antioxidant  
 434 capacity.



435 **Figure 10.** Effect of root imbibition by KIO<sub>3</sub> in the antioxidant capacity of tomato fruits and  
 436 leaves. Different letters indicate significant differences between treatments (Tukey HSD,  $p \leq$   
 437 0.05).  $n = 4$ .  
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#### 440 Antioxidant capacity of tomato fruits and leaves by Na<sub>2</sub>SeO<sub>3</sub> and KIO<sub>3</sub> interactions

441 The antioxidant capacity of the fruits by the hydrophilic ABTS method, presented differences  
 442 where the treatment of 3-250 mg L<sup>-1</sup>, presented a greater antioxidant capacity in comparison with the  
 443 treatment of 1 mg L<sup>-1</sup> of Na<sub>2</sub>SeO<sub>3</sub>, as well as with the control. The lipophilic ABTS method did not  
 444 present an effect between the treatments. The DPPH method presented a negative effect in the  
 445 treatments of 200 and 250 mg L<sup>-1</sup> of KIO<sub>3</sub>, which are the treatments with the lowest antioxidant  
 446 capacity in tomato fruits (Table 6).

447 In the leaves, the antioxidant capacity presented an effect through hydrophilic ABTS, where the  
 448 1-100 mg L<sup>-1</sup>, obtained a greater antioxidant capacity compared to the treatment of 0.5-100 mg L<sup>-1</sup>,  
 449 which was the treatment where there was a lower antioxidant activity in the leaves of the plant. In the  
 450 lipophilic ABTS method, there was an effect in the control, where there was a higher antioxidant  
 451 capacity compared to the treatment of 3-150 mg L<sup>-1</sup>, on the other hand, by means of hydrophilic  
 452 DPPH, an effect was presented in the 0.5-200 mg L<sup>-1</sup> treatment, where a greater antioxidant capacity  
 453 was obtained by this method, while the antioxidant capacity was affected with the use of 1 mg L<sup>-1</sup> of  
 454 Na<sub>2</sub>SeO<sub>3</sub> (Table 6).  
 455

456 **Table 6.** Effect of root imbibition by Na<sub>2</sub>SeO<sub>3</sub> and KIO<sub>3</sub> interactions in the antioxidant capacity  
 457 (µmol TE g<sup>-1</sup> DW) of tomato fruits and leaves.

Na <sub>2</sub> SeO <sub>3</sub> (mg L <sup>-1</sup> )	KIO <sub>3</sub> (mg L <sup>-1</sup> )	Fruto ABTS-H	Fruto ABTS-L	Fruto DPPH-H	Hoja ABTS-H	Hoja ABTS-L	Hoja DPPH-H
0	0	54.7bcdef	7.8 <sup>a</sup>	27.4ab	90.9cdef	20.4 <sup>a</sup>	34.9abcd
0	100	50.2cdef	2.0a	28.5ab	88.9cdef	17.0abc	33.0abcd
0	150	46.2defg	6.6 <sup>a</sup>	10.0b	81.6def	19.0ab	38.7abc
0	200	40.9efg	2.7 <sup>a</sup>	10.7b	131.4ab	11.5abcdef	30.4bcd
0	250	51.4cdef	1.9 <sup>a</sup>	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	8.4 <sup>a</sup>	24.3ab	11.3g	15.7abcd	30.1bcd
0.5	150	51.5bcdef	3.9 <sup>a</sup>	27.3ab	111.1abc	11.9abcdef	40.6ab
0.5	200	62.7abc	3.7 <sup>a</sup>	37.0a	104.5bcd	14.2abcdef	43.1 <sup>a</sup>
0.5	250	42.1efg	2.4 <sup>a</sup>	20.8ab	75.4ef	7.7bcdef	28.7bcd
1	0	31.1g	2.8 <sup>a</sup>	15.8ab	70.4f	12.4abcdef	23.0d
1	100	42.0efg	3.0 <sup>a</sup>	17.2ab	135.6 <sup>a</sup>	14.0abcdef	32.8abcd
1	150	67.9ab	4.4 <sup>a</sup>	25.5ab	105.7bc	14.6abcde	37.5abc
1	200	52.6bcdef	4.6 <sup>a</sup>	23.4ab	95.9cdef	11.9abcdef	40.5ab
1	250	54.3bcdef	2.7 <sup>a</sup>	27.7ab	74.9ef	12.9abcdef	39.4abc
2	0	59.0bcd	9.0 <sup>a</sup>	33.8 <sup>a</sup>	101.6cde	9.9abcdef	37.1abc
2	100	53.1bcdef	4.7 <sup>a</sup>	29.3ab	100.8cde	7.8bcdef	37.2abc
2	150	45.6defg	3.0 <sup>a</sup>	26.7ab	88.6cdef	6.8cdef	31.6abcd
2	200	50.6cdef	9.2 <sup>a</sup>	25.3ab	83.8cdef	3.5ef	36.7abc
2	250	52.4bcdef	5.1 <sup>a</sup>	27.4ab	91.3cdef	11.6abcdef	38.3abc
3	0	51.2cdef	3.9 <sup>a</sup>	29.9ab	82def	9.0abcdef	38.0abc
3	100	58.5bcd	6.7 <sup>a</sup>	26.0ab	92.3cdef	3.2ef	34.3abcd
3	150	50.3cdef	4.2 <sup>a</sup>	30.3ab	100.3cde	2.9f	35.2abc
3	200	51.7bcdef	5.2 <sup>a</sup>	32.5ab	16.1g	5.1def	33.0abcd
3	250	77.6 <sup>a</sup>	2.7 <sup>a</sup>	22.5ab	23.7g	10.4abcdef	28.2cd

Different letters within the columns indicate significant differences between treatments (Tukey HSD,  $p \leq 0.05$ ).  $n = 4$ .

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### Conclusions

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The application of  $\text{KIO}_3$  and  $\text{Na}_2\text{SeO}_3$  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.

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### Authors' Contributions

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

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### Ethical approval (for researches involving animals or humans)

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Does not apply.

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### Acknowledgements

476

Fernando Mejía-Ramírez gives thanks to the National Council of Humanities, Science and Technology of Mexico (CONAHCYT) for the financial support for doctoral studies.

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### Conflict of Interests

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The authors declare no conflict of interest.

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633 [%20Inventory%20of%20FV%20Inspection%20Aids.pdf](https://www.ams.usda.gov/sites/default/files/media/Official%20Inventory%20of%20FV%20Inspection%20Aids.pdf) (accessed 3.19.23).
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## CONCLUSIÓN GENERAL

En el presente trabajo de investigación se reporta que el uso de los tratamientos de imbibición en semillas como en raíz en plántulas con yodato de potasio y selenito de sodio en el cultivo de tomate presenta aspectos importantes en la concentración de compuestos antioxidante, sin embargo, en la forma de aplicación, por raíz presentó una inhibición en la actividad enzimática de las variables evaluadas, por lo tanto el establecimiento de una dosis óptima para este método es una alternativa que podría ser de utilidad para mejorar el estado fisiológico y bioquímico en el cultivo de tomate.



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