

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO
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APLICACIÓN FOLIAR DE NANOPARTÍCULAS DE COBRE EN PLANTAS DE
TOMATE SOMETIDAS A ESTRÉS SALINO

Tesis

Que Presenta ELSY RUBISELA LÓPEZ VARGAS
Como requisito parcial para obtener el Grado de
MAESTRO EN CIENCIAS EN HORTICULTURA

Saltillo, Coahuila

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Elaborada por ELSY RUBISELA LÓPEZ VARGAS como requisito parcial para
obtener el Grado de MAESTRO EN CIENCIAS EN HORTICULTURA con la
supervisión y aprobación del Comité de Asesoría




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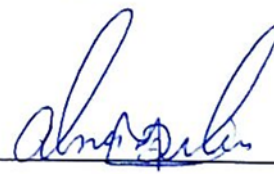
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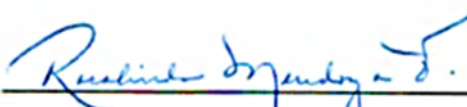
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A Dios, por permitirme continuar aquí, asombrándome cada vez más de su enorme energía.

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Carta de aceptación de Artículos

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Dear Authors,

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Subject: Plant Foods for Human Nutrition - Submission Notification to co-author
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To: Elsy Rubisela López-Vargas <lopez2690vargas@gmail.com>
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Re: "FOLIAR APPLICATION OF COPPER NANOPARTICLES INCREASES THE FRUIT QUALITY AND CONTENT OF BIOACTIVE COMPOUNDS IN TOMATO" Full author list: Elsy Rubisela López-Vargas; Hortensia Ortega-Ortiz; Gregorio Cadenas-Pliego; Karim de Alba Romenus; Adalberto Benavides Mendoza; Antonio Juárez-Maldonado

Dear MC Elsy López-Vargas,

We have received the submission entitled: "FOLIAR APPLICATION OF COPPER NANOPARTICLES INCREASES THE FRUIT QUALITY AND CONTENT OF BIOACTIVE COMPOUNDS IN TOMATO" for possible publication in Plant Foods for Human Nutrition, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr Antonio Juárez-Maldonado who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

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INTRODUCCIÓN

Hoy en día existe más interés por los alimentos que se consumen en la dieta humana, específicamente los compuestos bioactivos que están presentes en éstos (Hernández-Fuentes et al., 2017). Este tipo de compuestos ayudan a prevenir las enfermedades crónicas y degenerativas, además de generar efectos positivos para promover y restaurar las funciones fisiológicas del organismo humano, por lo tanto, es de suma importancia generar productos alimenticios saludables y de mejor calidad (Martí et al., 2018).

El fruto de tomate (*Solanum lycopersicum* L.) se ha identificado como un alimento funcional y nutracéutico (Adalid et al., 2010). Este fruto es una fuente importante de compuestos bioactivos como vitaminas, carotenoides y compuestos fenólicos, los cuales tienen gran actividad antioxidante que representa beneficios directos a la salud humana (Muzolf-Panek et al., 2017). Por lo tanto, el cultivo es de gran importancia para la dieta humana, además de que es la segunda hortaliza más consumida comercialmente después de la papa (Martínez et al., 2016).

Por otro lado, la salinidad es un problema agroambiental que limita el crecimiento y desarrollo de las plantas en las regiones áridas a semiáridas del mundo y se convierte en un problema grave (Abbasi et al., 2016). La magnitud del problema de la salinidad es de aproximadamente el 10% del área terrestre mundial y el 50% de las áreas irrigadas, lo que resulta en una pérdida de producción agrícola de 12 000 millones de dólares (Flowers et al., 2010).

Algunos procesos como la germinación, crecimiento y vigor vegetativo, y la generación de flores y frutos son afectados de manera adversa por altas concentraciones de sal, causando un rendimiento disminuido y una baja calidad de los productos (Taïbi et al., 2016). Además, se ha demostrado que induce la actividad de ciertas enzimas antioxidantes como la catalasa, la peroxidasa, la glutatión reductasa y la superóxido dismutasa para desintoxicar radicales libres generados en condiciones de estrés salino (Koleška et al., 2017).

Actualmente el progreso en la ciencia ha permitido el desarrollo de nuevas tecnologías para enfrentar los problemas presentes en la agricultura. Una de estas es la aplicación de la nanotecnología para mejorar la productividad de los cultivos (De la Rosa et al., 2017; Wang et al., 2016). En los últimos años se ha reconocido el potencial de la nanotecnología con la aplicación de nanomateriales como nanofertilizantes, nanopartículas, o nanopesticidas en el manejo de nutrientes, mejora genética, tratamiento de las enfermedades de las plantas y la promoción del crecimiento de éstas (De la Rosa et al., 2017). Éstos nanomateriales pueden facilitar una germinación más rápida de las plantas, o ayudar a las plantas a tolerar estrés biótico y abiótico, favorecen un manejo más eficiente de nutrientes, y aumentan el crecimiento de las plantas, lo que puede generar un impacto ambiental reducido en comparación con los métodos tradicionales (Genady et al., 2017).

Recientemente se ha investigado sobre el uso de nanopartículas (NPs) como insecticidas, fungicidas y nanofertilizantes (Raliya et al., 2016) con el objetivo reducir las cantidades aplicadas de productos fitosanitarios, minimizar las pérdidas de nutrientes en la fertilización e incrementar los rendimientos a través de un manejo optimizado de nutrientes (Das et al., 2015). Una concentración de NPs por encima de los rangos óptimos de metales como Zn, Cu, Ag, Ce, Ti, entre otros, produce estrés y/o toxicidad generando especies reactivas de oxígeno (EROs) lo que resulta en la disrupción del metabolismo celular (Aslani et al., 2014; Siddiqi y Husen, 2017).

Particularmente, el cobre es un micronutriente esencial asociado con los procesos biológicos de las plantas, desempeña un papel importante en el mantenimiento del metabolismo natural y el crecimiento de las plantas, sin embargo, en concentraciones por encima de sus niveles óptimos induce toxicidad en la planta (Tie et al., 2012).

Dosis bajas de estas NPs (5-20 mg Cu por planta), generan efectos metabólicos debido a la acumulación de Cu y la generación de EROs (Keller et al., 2017).

Se ha demostrado que las NPs Cu (0.006 mg L^{-1}) aplicadas al sustrato aumentan los fenoles totales y modifican la concentración de compuestos enzimáticos y no enzimáticos en los frutos de tomate (Juarez-Maldonado et al., 2016) y chile jalapeño con NPs Cu+Quitósán+PVA ($0-10 \text{ mg g}^{-1}$) aplicado en sustrato (Pinedo-Guerrero et al., 2017).

Existen muy pocos estudios sobre el efecto de las NP en plantas sometidas a estrés salino. Se ha reportado que las NP de ZnO en concentraciones de 15 a 30 mg L^{-1} tienen efectos positivos sobre el metabolismo de las plantas de tomate bajo estrés salino (Alharby et al., 2016). Del mismo modo, se ha demostrado que las concentraciones de $0,05$ a $2,5 \text{ mg L}^{-1}$ de Ag NP podrían mejorar la tolerancia de las plantas de tomate a la salinidad (Khan et al., 2017). En cultivo de canola, la aplicación de 200 y 1000 mg kg^{-1} de CeO₂ NPs mejoran el crecimiento y la fisiología de las plantas bajo estrés salino, pero no alivian completamente (Rossi et al., 2017).

En este sentido es importante estudiar los efectos de las NPs Cu sobre el cultivo de tomate a fin de poder hacer frente a la sintomatología presente en las plantas afectadas por estrés salino y comprender los procesos de tolerancia o resistencia que se generen con el fin de, en un futuro, poder utilizarlos para adaptar a los ambientes salinos a una mayor proporción de cultivos de interés económico para el hombre.

OBJETIVO GENERAL

Conocer las respuestas de inducción de la capacidad antioxidante y tolerancia al estrés salino en plantas de tomate tratadas con nanopartículas de cobre (NPs Cu) aplicadas foliarmente.

OBJETIVOS ESPECÍFICOS

- Evaluar el efecto de diferentes concentraciones de nanopartículas de cobre en plantas de tomate.
- Determinar la actividad enzimática y la capacidad antioxidante representativa en plantas de tomate tratadas con NPs Cu absorbidas foliarmente.

- Determinar la respuesta antioxidante, calidad poscosecha e inducción de tolerancia a estrés salino en plantas de tomate tratadas con NPs Cu aplicadas foliarmente.

HIPÓTESIS

Las nanopartículas de cobre aplicadas foliarmente pueden modificar la capacidad de adaptación de las plantas, aumentando la capacidad antioxidante y la inducción de tolerancia al estrés salino.

REVISIÓN DE LITERATURA

Cultivo del tomate

El tomate (*Solanum lycopersicum* L.) pertenece a la familia Solanaceae, es una planta hortícola anual con una importante distribución a lo largo del mundo y un gran valor económico, la cual presente una serie de efectos beneficiosos a través de su consumo debido a su elevado contenido en compuestos antioxidantes. Su consumo es en fresco, como ensalada, cocido o procesado en forma de pasta o ketchup de tomate (Manan et al., 2016). Aunque botánicamente el tomate es una fruta, sin embargo a efectos culinarios se le considera una hortaliza (Nuez y Díez, 2013).

Importancia nutricional

Recientemente, existe un gran interés público en atraer al consumidor hacia alimentos que pueden tener un efecto significativo sobre la salud humana, y que contienen unos niveles elevados de vitaminas, minerales y antioxidantes. Es ampliamente aceptado que una dieta saludable es un factor importante en la prevención de enfermedades crónicas como el cáncer y enfermedades cardiovasculares y neuro-degenerativas.

De un total de 40 carotenoides encontrados en la dieta humana, sólo 25 se encuentran en la sangre debido a la absorción selectiva del tracto digestivo. De este número, de 9-20 derivan del tomate tanto fresco como procesado, siendo los más importantes: licopeno, α - y β -caroteno, luteína, zeaxantina y β -criptoxantina. El licopeno, que constituye cerca del 80-90% del total del contenido en carotenoides de los tomates maduros es el antioxidante más eficiente entre los carotenoides por su actividad detoxificadora del oxígeno singlete y de los radicales peroxilo (Soares et al., 2017).

Por otro lado, la vitamina C además de ser esencial para la dieta humana se debe ingerir a partir de frutos ricos en ésta, ya que el cuerpo humano no tiene la capacidad de producirla (Padayatty et al., 2003) y específicamente en los frutos de tomate la vitamina C es de los compuestos más importantes (Martí et al., 2018), ya que permite actuar directamente como un antioxidante al secuestrar especies reactivas de oxígeno (EROs) previniendo o minimizando su daño (Gill y Tuteja, 2010).

El limitado aporte calórico, relativamente alto contenido en fibras, y la gran cantidad de minerales, vitaminas y fenoles como los flavonoides hacen del fruto de tomate un excelente “alimento funcional” que conlleva unos beneficios fisiológicos adicionales así como de requerimientos nutricionales básicos (Dorais et al., 2008).

Importancia económica

El tomate es la hortaliza más popular y difundida mundialmente, por lo tanto la de mayor valor económico. Su demanda aumenta continuamente en todo el mundo, y con ella su cultivo, producción y comercio. Con el incremento de la población mundial, también se ha incrementado el consumo de vegetales como parte de la dieta humana total. Su incremento anual en cuanto a producción en estos últimos años, se debe principalmente al aumento en el rendimiento y en menor proporción al aumento de la superficie cultivada.

Según la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), el tomate es el segundo vegetal más cultivado en el mundo, después de la papa, alcanzándose en el año 2014 valores de producción de aproximadamente 170.7 millones de toneladas de tomate fresco en todo el mundo, además destaca que el aumento en la producción mundial de tomate con respecto al año anterior ha sido de 7.031 millones de toneladas, un incremento del 4.29 %.

Entre los cinco países más productores del mundo encontramos a China, India, EEUU, Turquía y Egipto (FAOSTAT, 2014). México se encuentra ubicado en el lugar catorce con una producción mundial de 3,536 millones de toneladas. Según los datos de la ONU, en el pasado año 2016 se exportó en el mundo un total de 7,448.42 millones de toneladas de tomate, 0.65 % menos que el año anterior. México continúa liderando las exportaciones mundiales de tomate, con una cifra récord en 2016 de 1,748.86 millones de toneladas, un 12 % más que el año anterior.

La exportación mexicana de tomate es el 23.48 % del total mundial. Teniendo en cuenta los principales clientes de cada país, México destinó en 2016 la casi totalidad de sus exportaciones de tomate en fresco a los Estados Unidos, país al que vendió 1,743.89 millones de toneladas (HortolInfo, 2017).

Actualmente la producción nacional acumulada de tomate rojo en el país hasta septiembre 2017 es de 2 millones 100 mil toneladas, lo que representa 16.3% de incremento respecto de la obtenida el año previo. A nivel, nacional tres entidades concentran alrededor de 52% de la producción nacional, Sinaloa que se mantienen como el principal productor aporta 35% del total, seguida por San Luis Potosí, que produce 10%, y Zacatecas que genera 7%. La superficie total cosechada, a septiembre de 2017, fue de 34 mil hectáreas, mil 760 por encima de las obtenidas al mismo mes del año 2016 (SIAP, 2017).

Estrés salino

Los tomates son muy sensibles a los factores ambientales, como la temperatura, la luz y los cambios en el riego a lo largo del crecimiento de la planta. En el último siglo, el cultivo protegido ha alcanzado una enorme importancia, ya que abarca diversas técnicas de protección de plantas que van mucho más allá del simple riego.

En el cultivo en invernadero, debido a la alimentación suplementaria intensiva y la falta de lixiviación natural de la tierra, se produce un estrés salino, especialmente a altas temperaturas y alto consumo de agua por transpiración de las plantas (Pašalić et al., 2016). En general la presencia de sales solubles en el medio de cultivo afecta negativamente al desarrollo de las plantas de tres formas: i) disminuyendo el potencial hídrico del medio y restringiendo así la absorción de agua por las raíces (efecto osmótico), ii) por la absorción de iones salinos específicos, que puede determinar su acumulación en los tejidos en concentraciones que lleguen a ser tóxicas e induzcan desórdenes fisiológicos (toxicidad iónica específica) y iii) las concentraciones elevadas de iones salinos también pueden modificar la absorción de los nutrientes esenciales determinando desequilibrios nutricionales (efecto nutricional) (Shabala, 2012).

Bajo la salinidad, el déficit hídrico aparece como consecuencia del desequilibrio osmótico (Parida y Das, 2005) y altas concentraciones de Na^+ acumuladas, que en la solución del suelo pueden cambiar la relación $\text{Na}^+/\text{Ca}^{2+}$ o Na^+/K^+ .

El aumento de la producción de ROS (especies de oxígeno reactivo) o estrés oxidativo en las células vegetales es una consecuencia de la sequía fisiológica y el equilibrio de iones perturbado (Ahmad et al., 2008).

La mayor producción de peróxido de hidrógeno y superóxido ocurre con mayor frecuencia (Chawla et al., 2013), pero también se observa un incremento en la concentración de oxígeno singlete e hidroxil radicales (Esfandiari et al., 2007).

El estrés oxidativo puede conducir al daño oxidativo en todos los niveles celulares, comenzando desde el ADN hasta proteínas, lípidos y otras biomoléculas, que al final interrumpen la homeostasis celular y conducen a la muerte celular (Gill y Tuteja, 2010; Ahmad et al., 2011).

La salinidad disminuye el rendimiento del cultivo primero al reducir el crecimiento de los órganos que consumen asimilados y, en segundo lugar, al disminuir la producción de asimilación en los tejidos fuente fotosintéticamente activos (Hassan Numanman et al. 2013). La sensibilidad de la planta al estrés por sal varía significativamente según la especie, el órgano de la planta y la etapa de desarrollo, lo que también afecta la especificidad en las estrategias de defensa antioxidativa (Tester y Davenport, 2003; Maggio et al., 2004). Muchos investigadores han demostrado una correlación positiva entre la resistencia al estrés salino y la actividad de las enzimas de defensa antioxidantes (Sharma et al., 2012; Chawla et al., 2013; Gill et al., 2013; Gupta y Huang, 2014). La superóxido dismutasa (SOD, EC 1.15.1.1), la catalasa (CAT, EC 1.11.1.6), la ascorbato peroxidasa (APX, EC 1.11.1.11) y la peroxidasa de clase III (POD, EC 1.11.1.7) utilizan diferentes compuestos fenólicos como sustratos (ácido guayacol, cafeico, ferúlico o clorogénico) y representan una defensa antioxidante primaria (Sharma et al., 2012).

En estudios recientes del metabolismo antioxidante de la fruta de tomate, se ha demostrado que los cambios en la concentración de ascorbato y las actividades de las enzimas antioxidantes dependen del nivel de salinidad y la etapa de desarrollo de la fruta (Murshed et al., 2014).

El cobre

Particularmente, el cobre es un micronutriente esencial asociado con los procesos biológicos de las plantas, desempeña un papel importante en el mantenimiento del metabolismo natural y el crecimiento de las plantas, sin embargo, en concentraciones por encima de sus niveles óptimos induce toxicidad en la planta (Chen et al., 2015; Gui et al., 2012; Zhao et al., 2010).

Una absorción excesiva de Cu perturba los procesos celulares como la fotosíntesis, la respiración y el metabolismo de las paredes celulares, altera las estructuras proteicas, inactiva enzimas e inhibe el crecimiento de las plantas (Adrees et al., 2015; Cuypers et al., 2011; Mostofa y Fujita 2013; Zhao et al., 2010).

Un efecto significativo de la toxicidad del cobre es el estrés oxidativo, como metal redox activo, el Cu produce la formación EROs mediante las reacciones de Fenton-Haber-Weiss, que puede dañar moléculas y membranas mediante la inducción de la peroxidación lipídica (Angelé et al., 2017).

Nanotecnología en la agricultura

La tecnología de la que se dispone actualmente permite que existan diferentes alternativas para aumentar la producción y calidad nutraceútica de los cultivos hortícolas. Una de estas es la aplicación de la nanotecnología para mejorar la productividad (De la Rosa et al., 2017; Peng et al., 2015). En los últimos años se ha reconocido el potencial de la nanotecnología con la aplicación de nanomateriales como nanofertilizantes, nanopartículas, o nanopesticidas en el manejo de nutrientes, mejora genética, tratamiento de las enfermedades de las plantas y la promoción del crecimiento de éstas (De la Rosa et al., 2017). Éstos nanomateriales pueden facilitar una germinación más rápida de las plantas, o ayudar a las plantas a tolerar estrés biótico y abiótico, un manejo más eficiente de nutrientes, y el aumento del crecimiento de las plantas, lo que puede generar un impacto ambiental reducido en comparación con los métodos tradicionales (Genady et al., 2017). En la nanotecnología, una partícula se define como un objeto pequeño que se comporta como una unidad completa con respecto a su transporte y propiedades. Por lo tanto, el diámetro de partícula es una variable utilizada para clasificar esas partículas.

Por convención, las partículas gruesas son aquellas que cubren un rango de 10,000 a 2,500 nm, las partículas finas son aquellas que varían de 2,500 a 100 nm, y las nanopartículas (NP) (o partículas ultrafinas) tienen un tamaño de 1 a 100 nm, ya sea dispersos en medios gaseosos, líquidos o sólidos (Capaldi et al., 2015).

En este sentido, se destaca el papel de las nanopartículas en la mejora de la seguridad del suelo, los diferentes efectos sobre las plantas y la importancia de estas nanopartículas para la nutrición de las plantas (Shalaby et al., 2016). Recientemente se ha investigado sobre el uso de nanopartículas (NPs) como insecticidas, fungicidas y nanofertilizantes (Raliya et al., 2016) con el objetivo reducir las cantidades aplicadas de productos fitosanitarios, minimizar las pérdidas de nutrientes en la fertilización e incrementar los rendimientos a través de un manejo optimizado de nutrientes (Das et al., 2015).

Se ha demostrado que el impacto de las NPs en las plantas depende de muchos factores como la composición, concentración, tamaño, y las propiedades físicas y químicas, e inclusive la especie vegetal de estudio (Shalaby et al., 2016). Una concentración de NPs por encima de los rangos óptimos de metales como Zn, Cu, Ag, Ce, Ti, entre otros, produce estrés y/o toxicidad generando especies reactivas de oxígeno (EROs) lo que resulta en la interrupción del metabolismo celular. Bajo estas condiciones, las plantas producen enzimas antioxidantes y componentes no enzimáticos que protegen el sistema celular y subcelular de los efectos citotóxicos de las EROs (Aslani et al., 2014; Siddiqi y Husen, 2017).

Nanopartículas de Cobre

A diferencia del Cu a granel, las NPs de Cu tienen propiedades ligadas a su bajo tamaño y alta superficie específica que les confieren características diferentes, como resistencias físicas, reactividad química, conductividad eléctrica, magnetismo y efectos ópticos en comparación con el material de cobre a granel (Hong et al., 2015).

Dosis bajas de estas NPs (5-20 mg Cu por planta), generan efectos metabólicos debido a la acumulación de Cu y la generación de EROs (Keller et al., 2017).

Hong et al., 2015 describieron que las NPs Cu (0-20 mg L⁻¹) aplicadas vía riego no sólo reducían el crecimiento de lechuga y la alfalfa, sino que también alteraban el contenido nutricional y la actividad enzimática.

Lee et al., 2008 reportaron que la longitud de las plántulas de frijol Mung y trigo se redujeron en un 60% y 75%, respectivamente, cuando se expusieron a 1000 mg L⁻¹ de NPs Cu aplicadas en medio agar. NPs de CuO (0-500 mg L⁻¹) aplicadas en medio Murashige y medio Skoog redujo el crecimiento de soya (Nair y Chung, 2014a).

Así mismo las NPs de CuO (0-100 mg L⁻¹) aplicadas en los mismos medios mostraron una reducción significativa en la biomasa vegetal y en el contenido total de clorofila con una concentración superior a 2 mg L⁻¹ en *Arabidopsis thaliana*. Sin embargo, hubo un incremento en flavonoides a concentraciones de 5 mg L⁻¹. También mostró una inducción significativa de genes relacionados con las respuestas al estrés oxidativo, la asimilación de azufre, el glutatión y la biosíntesis de prolina bajo el estrés de NPs de CuO (Nair y Chung, 2014b).

Las NPs de CuO (0-2000 mg L⁻¹) aplicadas vía riego inhibieron significativamente el crecimiento de algodón transgénico y convencional a concentraciones superiores a 10 mg L⁻¹ (Le et al., 2014). Mientras que las NPs CuO (0-200 mg L⁻¹) aplicadas foliarmente en plantas de pepino redujeron significativamente la firmeza del fruto comparado con el control a excepción de la concentración de 100 mg L⁻¹ y a 200 mg L⁻¹ redujo el Mo de la fruta en 51% comparado con el control (Hong et al., 2016).

Además se ha demostrado que las NPs de Cu (0.006 mg L⁻¹) aplicadas al sustrato aumentan los fenoles totales y modifican la concentración de compuestos enzimáticos y no enzimáticos en el cultivo de tomate (Juarez-Maldonado et al., 2016) y en chile jalapeño con NPs Cu+Quitósán+PVA (0-10 mg g⁻¹) aplicado en sustrato (Pinedo-Guerrero et al., 2017).

Existen muy pocos estudios sobre el efecto de las NP en plantas sometidas a estrés salino. Se ha reportado que las NP de ZnO en concentraciones de 15 a 30 mg L⁻¹ tienen efectos positivos sobre el metabolismo de las plantas de tomate bajo estrés salino (Alharby et al., 2016).

Del mismo modo, se ha demostrado que las concentraciones de 0,05 a 2,5 mg L⁻¹ de Ag NP podrían mejorar la tolerancia de las plantas de tomate a la salinidad (Khan et al., 2017). En cultivo de canola, la aplicación de 200 y 1000 mg kg⁻¹ de CeO₂ NPs mejoran el crecimiento y la fisiología de las plantas bajo estrés salino, pero no alivian completamente (Rossi et al., 2017).

La producción de compuestos antioxidantes (GSH, vitamina C y los carotenoides) incluyendo a las enzimas antioxidantes (APX, SOD y CAT) se activa en las plantas para disminuir el estrés oxidativo causado por las EROs (Caverzan et al., 2012; Shaw et al., 2014; Shaw y Hossain, 2013).

Esta activación de las enzimas antioxidantes y la síntesis de compuestos antioxidantes son factores clave para la tolerancia a diferentes tipos de estrés abiótico en las plantas (De la Rosa et al., 2017; Keller et al., 2017). Además, de que se tiene como beneficio adicional la acumulación de compuestos bioactivos en los cultivos, lo que representa beneficios directos para la salud humana.

Article

Postharvest Behavior of Bioactive Compounds in Tomato Fruits Treated with Cu Nanoparticles and NaCl Stress

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Abstract: Tomatoes are important for human diet due to their content of bioactive compounds. However, is little known about behavior of these compounds during fruit shelf life. The goal of this research was to evaluate the effects on bioactive compounds of tomato fruits stored during different times and conditions, obtained from tomato plants developed under conditions of saline stress and with the application of copper nanoparticles. Four treatments were evaluated: foliar spray of copper nanoparticles (250 mg L⁻¹) with or without saline stress, only saline stress, and the absolute control. The results show that application of copper nanoparticles has a positive effect on the accumulation of bioactive compounds such as total phenols, β -carotene, and vitamin C. The saline stress during the development of tomato plants causes a decrease of the bioactive compounds as well as antioxidant capacity in tomato fruits. However, this negative effect can be reduced with the application of copper nanoparticles. The application of copper nanoparticles may be a technique to increase and maintain the content of bioactive compounds in tomato fruits and can be an effective alternative to diminish the negative effects on bioactive compounds caused by saline stress.

Keywords: antioxidant capacity; saline stress; storage conditions; temperature effect

1. Introduction

Tomatoes (*Solanum lycopersicum* L. from the Solanaceae family) are among the most consumed vegetables in the world, either fresh or as processed products [1]. Tomatoes are important in the human diet because they contain different bioactive compounds such as phenolic compounds, vitamin C, and lycopene [2]. Among these compounds are the carotenoids, which are responsible for the coloration of the fruits during ripening [1]. Lycopene is the most abundant carotenoid in tomato fruits and accounts for more than 80% of total carotenoids [3]. β -carotene is the second most abundant carotenoid [1] and together with lycopene make up the majority of carotenoids present in tomato fruits. Vitamin C is the most important vitamin in fruits and vegetables for human nutrition: more than 90% of vitamin C in the human diet comes from these sources [4]. Particularly in tomato fruits, vitamin C is one of the most important bioactive compound, in addition to being a potent antioxidant [1]. Bioactive compounds such as lycopene, total phenols, vitamin C, carotenoids, and total flavonoids are very important because of their biological and physico-chemical properties, especially as antioxidant compounds and for the benefits they represent in human health [3].

On the other hand, it is known that saline stress affects a wide variety of crops worldwide: it is reported that over 6% of the world's land is affected by salinity [5]. Some of the effects of saline stress on plants is that it reduces the rate of expansion of the foliar surface, the water potential and osmotic tend to be more negative, the thickness of the epidermis and the mesophyll increases, the levels of Na and Cl increases, it decreases Ca, K, and Mg levels, and induces the activity of certain antioxidant enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase [6]. In addition, it is well known that saline stress affects a great diversity of crops, causing negative effects as the reduction of the rate of expansion of the leaf surface. The water and osmotic potential decrease, the levels of Na and Cl increase, and the contents of Ca, K, and, Mg decrease. In addition, it induces the activity of certain antioxidant enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase [6].

This stress can directly modify the quality of the fruits, especially the content of bioactive compounds during post-harvest life. However, progress in science has allowed the development of new tools for agriculture. Among these technologies, nanotechnology stands out, which generates great expectations for the development of new applications in a wide range of industrial sectors, and of human nutrition. A complete revolution is expected in the food industry, from food production, processing, and storage of vegetables and other products [7]. However, the application of nanotechnology to plant science has received less interest compared to other areas such as nanomedicine or nanopharmacology [8]. Even so, the application of various nanoparticles (NPs) has been evaluated in some crops, in which different results have been reported due to the species of plants used, as well as the dose and type of NPs [9]. Copper is a microelement necessary for plant development. This microelement takes part as a structural element in protein regulation; participates in photosynthetic electron transport, mitochondrial respiration, cell wall metabolism, hormone signaling, and oxidative stress response; is a cofactor for many enzymatic reactions carried out by enzymes such as polyphenol oxidase, amino oxidase, plastocyanin, laccase, and super oxide dismutase; and at the cellular level, it plays an important role in oxidative phosphorylation, signal trafficking machinery, and iron mobilization [10]. Meanwhile, copper nanoparticles have been some of the most evaluated nanoparticles: it has been shown that when applied at low concentrations in seeds or soil, it improves plant growth and increases the content of chlorophyll, phenolic compounds, and some enzymes such as CAT, SOD, and PAL [2,11-13]. Cu NPs are considered to have stimulatory effects to induce antioxidant compounds [9]. The nanoparticles can cross cell walls [10] by several ways: endocytosis, pore formation, carrier proteins, or through plasmodesmata [14]. These nanoparticles can interact with the intracellular structures [10], stimulating the formation of ROS, which in turn activates the plant defense system generates enzymatic and non-enzymatic antioxidant compounds [9]. It has been reported in maize that CuO NPs are traslocated from roots to shoots in hydroponic culture and cause root and shoot biomass decreases [15]. However, few long-term studies have shown the potential toxicity of NPs over the complete life cycle of the plants [16]. Moreover, the Cu NPs have a direct effect on fruit quality during storage. It has been demonstrated that application of these NPs increase pH and lycopene in tomato fruits [2] and diminish weight loss in jalapeño pepper [11]. This positive effect can be observed under light stress conditions by NPs, but can change under conditions of high stress where the activity of the antioxidant enzymes decreases due to the oxidative explosion [9]. Thus, it is possible to find different effects on antioxidant capacity depending on the dose of NPs used. There are very few studies on the effect of NPs on plants subjected to saline stress. Some studies report that ZnO NPs in concentrations of 15 to 30 mg L⁻¹ have positive effects on the metabolism of tomato plants under saline stress [17]. Likewise, it has been shown that concentrations of 0.05 to 2.5 mg L⁻¹ of Ag NPs could improve the tolerance of tomato plants to salinity [18]. In canola crop, application of 200 and 1000 mg kg⁻¹ of CeO₂ NPs improved the growth and physiology of plants under saline stress but did not completely alleviate it [19]. Although the use of NPs in conjunction with salinity stress has been studied, few investigations consider the effect on the behavior of the bioactive compounds in the post-harvest life of the fruits. Recently, the effect of the application of Cu NPs on PVA chitosan

hydrogels in certain post-harvest characteristics of the jalapeño pepper was studied [11]. However, the analysis of the bioactive compounds was not considered when the fruits were stored. Although the effect of Cu NPs on several crops has been studied, it is of utmost importance to study the postharvest life of products that serve as food for humans, since they directly influence human health. In addition, the effect of saline stress on the quality of food produced should also be considered. Therefore, the objective of this work was to evaluate the effects on bioactive compounds of tomato fruits stored during different times and conditions, obtained from tomato plants developed under conditions of saline stress and with the application of copper nanoparticles.

2. Materials and Methods

2.1. Crop Development

The experiment consisted in the establishment of tomato "Huno F1" (Harris Moran, Davis, CA, USA) saladette type of indeterminate growth habit. The transplant was carried out 36 days after sowing in black polyethylene bags with a capacity of 10 L. The crop was managed on a single stem. It was developed for 100 days from transplanting until obtaining fruits to harvest. A mixture of perlite-peat moss in a 1:1 ratio (base on volume) was used as the substrate. For irrigation, a directed drip irrigation system was used. Steiner's nutrient solution [20] was used for crop nutrition. The pH of the nutrient solution was adjusted with sulfuric acid to 6.5 each time the nutrient solution was prepared.

2.2. Treatments

For the experiment, the following treatments were considered: plants with stress by NaCl (T1), plants without stress and with application Cu nanoparticles at 250 mg L⁻¹ (T2), plants with stress by NaCl and with application Cu nanoparticles at 250 mg L⁻¹ (T3), and an absolute control without application of Cu nanoparticles and without stress by NaCl (T0). For treatments under stress, sodium chloride (NaCl) was applied to the nutrient solution at a concentration of 50 mM NaCl. This was done throughout the development of the crop. For treatments that included the application of Cu nanoparticles (Cu NPs), two foliar applications were performed at 57 and 78 days after transplantation (dat), coinciding with the mooring and fruit set, respectively. In the first application of Cu NPs, approximately 25 mL per plant was used, evenly spraying the whole plant. In the second application, about 35 mL per plant was sprayed following the same method. Both applications added a total of 50 mL per plant at a concentration of 250 mg L⁻¹ of Cu NPs, so that the total application was 12.5 mg of Cu NPs per plant. The applied copper nanoparticles were synthesized at the Applied Chemistry Research Center following the methodology described by [21]. The Cu NPs used have an average size of 50 nm and spherical shapes.

2.3. Reagents

Phenolphthalein, sodium hydroxide, aluminum chloride hexahydrate, sodium nitrite, sodium chloride, sulfuric acid, potassium sulfate, cupric sulfate, sodium carbonate, metaphosphoric acid, potassium persulfate, chloroform, hexane, methanol, and acetone purchased from JT Baker (Avantor Performance Materials, Center Valley, PA, USA). Gallic acid, 2,6-dichlorophenol-indophenol, 2,2^t-diphenyl-1-picrylhydrazyl (DPPH), trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), citric acid, rutin, and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Chemistry SA Of C.V (St. Louis, MO, USA). All aqueous solutions were prepared with Milli-Q[®] filtered water (resistivity > 18 cm MU) (Millipore, Bedford, MA, USA).

2.4. Samples Preparation

At 100 days after transplantation, the tomato fruits were harvested. In order to carry out the corresponding analyzes, the fruits were selected verifying that they were not physically damaged,

were uniform, and in maturity 6 (light red) according to the visual color pattern used by the United States Department of Agriculture [22]. These fruits were washed and used as whole for the analysis.

A total of 24 fruits were taken per treatment. Twelve of these were kept at room temperature (24 ± 1 °C), while the rest were placed under refrigeration at a constant temperature of 4 ± 1 °C and relative humidity of 80%. The bioactive compounds in these fruits were evaluated at the time of harvest (day 0) and later at 8, 15, and 23 days of storage (ds).

For the evaluation of bioactive compounds considering dry weight, the fruits of each evaluation time were subjected to deep freezing at -70 °C (THERMO SCIENTIFIC 303 Ultra Freezer). They were then lyophilized in a LABCONCO freeze dryer (LABCONCO, Model 79480, Kansas City, MO, USA) at a vacuum pressure of 133×10^{-3} mBar and a temperature of -40 °C. After freeze-drying, the fruits were ground in a knife mill (RTSCH GM 200, Haan, Germany) at 9000 rpm for 50 s until a fine powder of 150 microns was obtained. The samples were packed and storage in hermetic bags at 5 °C for two days. After this time, the samples were processed.

2.5. Total Phenols

Total phenols were determined by the Folin-Ciocalteu method described by [23]. The sample (0.5 g) was extracted with 10 mL of 80% methanol. The mixture was vortexed for 20 min. The tubes were centrifuged (Thermo Scientific Mod. ST 16R centrifuge, Langenselbold, Germany) at $15,000 \times g$ for 10 min. The supernatant was recovered, from which 0.5 mL was taken, and 5 mL of the 50% Folin reagent diluted with distilled water was added. It was allowed to stand for 7 min, then 4 mL of 7.5% sodium carbonate was added and placed in complete darkness for 1 h. Subsequently, the absorbance was read at 725 nm in a spectrophotometer (model 6715 UV/Vis, Jenway, Techne Inc., Burlington, NJ, USA) using methanol as a blank. A calibration curve was made with a standard solution of gallic acid at a concentration of 1000 mg L^{-1} . The results are expressed in equivalent milligrams of gallic acid per 100 g dry weight ($\text{mg EGA } 100 \text{ g}^{-1} \text{ DW}$).

2.6. Flavonoids

Flavonoids were determined according to the methodology of [24]. The sample (0.5 g) was mixed with 10 mL of pure methanol, vortexed for 2 min, and centrifuged in a centrifuge (Thermo Scientific, Model ST 16R, Germany) at $12,000 \times g$ for 10 min. Then, 0.5 mL of the extract was taken, and 0.15 mL of 5% NaNO_2 was added and allowed to stand for 5 min in the dark. Subsequently, 0.15 mL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and 1 mL of NaOH were added and allowed to stand for 15 min. Absorbance was read at 415 nm in a spectrophotometer (Model 6715 UV/Vis, Jenway, Techne Inc, USA). Total flavonoid content was determined using a standard quercetin curve. The results were expressed in milligrams equivalent of quercetin per 100 g dry weight ($\text{mg EQ } 100 \text{ g}^{-1} \text{ DW}$).

2.7. Carotenoids

Lycopene and β -carotene were determined by the method described by [25]. The sample (0.1 g) was mixed with 20 mL of hexane:acetone solution (3:2). An aliquot was taken from the supernatant and measured at 453, 505, 645, and 663 nm in a spectrophotometer (model 6715 UV/Vis, Jenway, Techne Inc., USA). The content of lycopene and β -carotene was estimated using the following equations:

$$\text{Lycopene} = -0.0458 A_{663} + 0.204 A_{645} + 0.372 A_{505} - 0.0806 A_{453}$$

$$\beta\text{-carotene} = 0.216 A_{663} - 1.220 A_{645} - 0.304 A_{505} + 0.452 A_{453}$$

The results were expressed in milligrams per 100 g fresh weight ($\text{mg } 100 \text{ g}^{-1} \text{ FW}$).

2.8. Ascorbic Acid

The determination of ascorbic acid was performed by the method of [26]. The sample (0.1 g) was mixed with metaphosphoric acid (0.1 g L^{-1}) for 45 min at $4 \text{ }^{\circ}\text{C}$ under constant stirring. It was then centrifuged at $15,000 \times g$. From the supernatant was taken 1 mL and mixed with 2,6-dichlorophenol-indophenol acid. The absorbance was read at 515 nm. The results were expressed as milligrams of ascorbic acid per 100 g fresh weight ($\text{mg AA } 100 \text{ g}^{-1} \text{ FW}$).

2.9. Antioxidant Activity by ABTS and DPPH

The antioxidant activity was determined by the method developed by [27], free radical detoxifier 2,2-diphenyl-1-picrylhydrazyl (DPPH), and by the Trolox equivalent antioxidant capacity method (TEAC) described by [28].

The sample (0.1 g) was mixed with 10 mL of methanol and centrifuged at $15,000 \times g$ for 10 min at $5 \text{ }^{\circ}\text{C}$. For the DPPH method, 0.3 mL of the methanolic extract was taken and 2.7 mL of a cold methanolic solution with DPPH was added ($6 \times 10^{-5} \text{ M}$). This was left to stand in complete darkness for 60 min at $4 \text{ }^{\circ}\text{C}$. The absorbance was then read at 515 nm. All results of antioxidant activity DPPH were expressed in μM Trolox equivalents per gram of fresh weight.

For the TEAC method, the ABTS $\bullet+$ radical was obtained by reacting ABTS [2,2^t-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid)] (7 mM) with potassium persulfate (2.45 mM) incubated at room temperature ($24 \text{ }^{\circ}\text{C}$) and in darkness for 16 h. Once the ABTS $\bullet+$ radical was formed, it was diluted with methanol to an absorbance value of 0.700 (± 0.1) at 734 nm. The methanolic extract of tomato and diluted ABTS $\bullet+$ was mixed, and after 6 min, its absorbance was read at 734 nm. All results of antioxidant activity ABTS were expressed in μM Trolox equivalents per gram of freshweight.

2.10. Statistical Analysis

A completely randomized design with three replicates per treatment was used. Statistical Analysis System v9.1 was used for the statistical analysis. An analysis of variance and Fisher's least significant difference test ($p \leq 0.05$) was performed.

3. Results and Discussion

The total phenols content in tomato fruits was statistically different between treatments at all evaluation times and for both storage conditions (Table 1). At the time of harvest, the treatment without stress and with application of Cu NPs presented the highest content of total phenols, whereas the T0 presented the lowest content.

Table 1. Total phenols content (milligrams equivalent of gallic acid per 100 g dry weight) in tomato fruits at different storage times at room temperature ($24 \pm 1 \text{ }^{\circ}\text{C}$) and refrigeration ($4 \pm 1 \text{ }^{\circ}\text{C}$ and relative humidity at 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	$35.69 \pm 0.40 \text{ a}$	$30.90 \pm 0.75 \text{ b}$	$37.88 \pm 0.60 \text{ a}$	not done
	NaCl	$35.14 \pm 0.86 \text{ ab}$	$26.81 \pm 0.71 \text{ d}$	$33.64 \pm 0.60 \text{ f}$	not done
	NPs Cu + NaCl	$34.94 \pm 0.91 \text{ ab}$	$29.50 \pm 0.58 \text{ c}$	$35.14 \pm 0.48 \text{ de}$	not done
	T0	$34.19 \pm 0.65 \text{ b}$	$32.60 \pm 0.71 \text{ a}$	$36.83 \pm 0.60 \text{ b}$	not done
Refrigeration	NPs Cu	not done	$31.25 \pm 1.14 \text{ b}$	$36.63 \pm 0.46 \text{ bc}$	$38.08 \pm 0.78 \text{ a}$
	NaCl	not done	$30.75 \pm 0.65 \text{ b}$	$34.84 \pm 0.35 \text{ e}$	$35.04 \pm 0.69 \text{ bc}$
	NPs Cu + NaCl	not done	$31.35 \pm 0.54 \text{ b}$	$35.74 \pm 0.60 \text{ cde}$	$33.89 \pm 0.65 \text{ c}$
	T0	not done	$32.89 \pm 0.57 \text{ a}$	$35.94 \pm 0.71 \text{ bcd}$	$36.29 \pm 0.54 \text{ b}$

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L^{-1} . NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L^{-1} . T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

At eight days, the fruits of the T0 plants had the highest total phenol content for both storage conditions. In addition, the fruits of plants with saline stress stored at room temperature had the lowest content. However, the application of Cu NPs in plants with saline stress had a higher content of phenols in fruits than those of plants with NaCl alone. This suggests that the application of Cu NPs decreases the adverse effect of NaCl on fruits, avoiding the loss of total phenols. In addition, refrigeration also has this effect, since treatments including both NaCl and Cu NPs are not different from each other.

At 15 days of storage, the fruits of the plants with application of Cu NPs presented the highest contents of total phenols for both storage conditions. In contrast, the fruits of plants with saline stress had the lowest total phenol content in both cases. It was also observed that the application of Cu NPs in plants with NaCl stress induced higher content of total phenols in fruits compared to plants that were only under saline stress. This corroborates that the application of Cu NPs reduces the adverse effect caused by saline stress, reducing the loss of total phenols in tomato fruits especially when stored at room temperature.

Finally, at 23 days of refrigerated storage, it was observed that the fruits of the plants with application of Cu NPs presented the highest total phenols content. This indicates that the application of Cu NPs has a positive effect on the total phenols content in tomato fruits and on the conservation of these compounds through storage time. Total phenols are antioxidants that trigger a series of secondary metabolites synthesized through the pathway of the shikimic acid or malonic acid, exerting cellular signaling functions under conditions of abiotic stress [29]. The main phenolic compounds found in tomato are the flavonoids rutin, naringenin, naringenin chalcone, and quercetin and the hydroxycinnamic acids chlorogenic and caffeic acids [1]. However, these exist in different concentration in the fruits due to the structural diversity among the phenolic compounds [3]. These compounds give the antioxidant capacity in the fruits due to the reduction of oxidative changes in cells by reducing the levels of free radicals [3]. It is suggested that Cu NPs have a stimulatory effect on the induction of antioxidant compounds derived from stress within the cells [11]. This may result in the formation of phenols as observed in this work.

The phenolics derived from aromatic amino acids and their precursors are some of the very wide range of compounds derived from shikimic acid. These compounds are a group of structurally diverse plant secondary metabolites that include terpenoids, phenylpropanoids, cinnamic acids, lignin precursors, hydroxybenzoic acids, catechols, coumarins, flavanoids, isoflavanoids, and tannins [30]. These varieties of metabolites are very important in stress tolerance by plants [3], and their accumulation are a good response in plants.

The content of flavonoids in tomato fruits was also statistically different between treatments at all evaluation times and for both storage conditions (Table 2). At the time of harvest, the fruits of the plants with saline stress had the highest content of flavonoids, while the fruits of the plants with Cu NPs and NaCl had the lowest content. Apparently, salinity stress increases this type of compound in fruits. However, this changes with the application of Cu NPs.

At eight days of storage, the opposite was observed, for storage at room temperature and in refrigeration the fruits of the plants with application of Cu NPs and NaCl had the highest content of flavonoids. It was also observed that in both cases, the fruits of the T0 plants had the lowest content of these compounds.

At 15 days of storage, a significant decrease in flavonoid content was observed in all treatments except for T0 under refrigeration. At room temperature, the T0 also had the highest flavonoid content. In addition, for both storage conditions, the fruits of the plants with NaCl had the lowest flavonoid content. This indicates that salinity stress negatively affects the flavonoid content when fruits are stored for 15 days.

Finally, at 23 days of storage under refrigeration, all treatments except for T0 increased the flavonoid content compared to the previous evaluation. In this case, the fruits of the plants with NaCl followed by the fruits of the plants with application of Cu NPs presented the highest content of

flavonoids. Flavonoids are a large and diverse group of low molecular weight polyphenolic secondary metabolites in plants [3]. Flavonoids are the major phenolic compounds found in tomato [1,31] and are also related to stress responses [3]. Therefore, it is expected that stress by NaCl or the application of Cu NPs will induce an effect on flavonoid activity [32]. The total flavonoid content decreases during fruit maturation [33]. Therefore, it is normal to observe a decrease as the storage time increases. In addition to this, the effect of refrigeration can reduce this effect. Therefore, under this condition, it is normal to observe a higher concentration of flavonoids. Pinedo-Guerrero et al. [11] reported that the higher content of flavonoids in jalapeño chili fruits was observed under refrigeration conditions. The lack of a precise response in flavonoids over storage time is normal. Each specific flavonoid compound may have different roles as well as unique patterns of accumulation and degradation [31]. This results in different storage trends across storage time. Although flavonoids are a type of phenolic compounds, they are so variable that tomato plants can respond differently and generate different amounts of groups of metabolites when they are exposed to some stress [3]. This results in that there is not necessarily a correlation between flavonoids and total phenols. This is observed in the results of the present study (Tables 1 and 2) as well as in other studies in tomato [1,31,34] and jalapeño pepper [11].

Table 2. Flavonoid content (milligrams equivalent of quercetin per 100 g dry weight) in tomato fruits at different storage times at room temperature (24 ± 1 °C) and refrigeration (4 ± 1 °C and relative humidity of 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	109.10 \pm 1.60 ab	123.28 \pm 2.54 b	37.78 \pm 3.00 bc	not done
	NaCl	113.34 \pm 2.54 a	106.56 \pm 2.57 c	20.00 \pm 3.67 g	not done
	NPs Cu + NaCl	81.38 \pm 2.54 c	142.96 \pm 3.86 a	32.91 \pm 0.97 cd	not done
	T0	107.83 \pm 3.00 b	100.21 \pm 3.88 d	37.99 \pm 2.64 b	not done
Refrigeration	NPs Cu	not done	118.42 \pm 2.23 b	27.41 \pm 2.91 ef	86.88 \pm 1.94 b
	NaCl	not done	118.63 \pm 1.60 b	23.39 \pm 2.64 fg	94.71 \pm 1.90 a
	NPs Cu + NaCl	not done	138.73 \pm 1.32 a	29.74 \pm 3.83 de	79.26 \pm 3.61 c
	T0	not done	105.51 \pm 3.81 c	109.53 \pm 2.64 a	82.01 \pm 2.77 bc

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L⁻¹. NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L⁻¹. T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

The content of lycopene in tomato fruits presented statistical differences between treatments at all times of evaluation (Table 3). At the time of harvest, the highest lycopene content was present in the fruits of the T0 plants. While the lowest content was found in the fruits of plants with NaCl.

Table 3. Lycopene content (milligrams per 100 g fresh weight) in tomato fruits at different storage times at room temperature (24 ± 1 °C) and refrigeration (4 ± 1 °C and relative humidity of 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	5.20 \pm 0.02 b	8.09 \pm 0.03 c	9.11 \pm 0.01 b	not done
	NaCl	3.87 \pm 0.02 d	7.73 \pm 0.03 d	9.99 \pm 0.03 a	not done
	NPs Cu + NaCl	4.68 \pm 0.02 c	8.36 \pm 0.01 b	8.00 \pm 0.01 c	not done
	T0	5.24 \pm 0.01 a	8.84 \pm 0.02 a	5.54 \pm 0.02 g	not done
Refrigeration	NPs Cu	not done	6.92 \pm 0.02 e	4.59 \pm 0.03 h	6.97 \pm 0.03 c
	NaCl	not done	3.73 \pm 0.02 h	5.99 \pm 0.02 e	7.02 \pm 0.02 b
	NPs Cu + NaCl	not done	4.07 \pm 0.01 g	5.68 \pm 0.01 f	6.71 \pm 0.03 d
	T0	not done	6.04 \pm 0.02 f	7.00 \pm 0.02 d	7.07 \pm 0.01 a

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L⁻¹. NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L⁻¹. T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

After storage for eight days, the fruits of all treatments at room temperature showed a significant increase in the lycopene content compared to the evaluation at the time of harvest. This is probably due to the natural process of fruit ripening [35]. In addition, the fruits of the treatments under refrigeration had lower contents of lycopene compared to the fruits stored at room temperature. This is due to the fact that the low storage temperature decreases the maturation processes of the fruits and as a result also reduces their accumulation of lycopene. However, for both storage conditions, the fruits of the plants with NaCl presented the lowest contents of lycopene. After 15 days of storage, the behavior of the fruits of the different treatments evaluated was completely different from the previous evaluation. Under conditions of room temperature, the fruits of the T0 plants presented the lowest content of lycopene, while the fruits of the plants with NaCl were the ones with the highest content. The fruits of the rest of the treatments were also superior to the T0 under this condition. On the other hand, under refrigeration, the fruits of the T0 plants showed the highest content of lycopene, while the fruits of the plants with application of Cu NPs had the lowest content.

After 23 days of storage under refrigeration, the fruits of the T0 plants had the highest content of lycopene followed by the fruits of the plants with NaCl. In this case, the fruits of the plants with Cu NPs and NaCl presented the lowest lycopene content. Lycopene is the most abundant carotenoid in tomato fruits, in addition to being one of the main antioxidants [1,3]. It has previously been shown that Cu NPs in Cs-PVA hydrogels when applied to the substrate increase the lycopene content in tomato [2]. Other studies have shown that foliar and in soil application of ZnO NPs and TiO₂ NPs increased lycopene content in tomato [36]. This was not consistently observed in the present study, possibly due to the high stress condition. Under conditions of high stress, an oxidative burst can be generated resulting in the opposite effect [9].

The β -carotene content in tomato fruits was different between treatments at all evaluation times (Table 4). At the time of harvest, the fruits of the plants with application of Cu NPs presented the highest content of β -carotene, while the fruits of the plants corresponding to the treatment with salinity had the lowest content. The fruits of the plants with application of Cu and NaCl NPs were also superior to the fruits of the plants with NaCl, indicating a beneficial effect of the application of Cu NPs mitigating the negative effect of salinity. At eight days of storage once more the fruits of the plants with application of Cu NPs were the ones with higher contents of β -carotene at room temperature along with the fruits of the plants with application of Cu NPs plus NaCl. It was also observed that the fruits of the plants with salinity had the lowest content of this β -carotene same as the previous evaluation. In refrigeration, the fruits of the T0 plants along with the fruits of the plants with application of Cu NPs presented the highest content of β -carotene. This indicates that the stress caused by the application of NaCl significantly decreases the β -carotene content in tomato fruits after eight days of storage regardless of the temperature condition. At 15 days of storage, regardless of temperature, all treatments showed an increase in β -carotene content. At room temperature, the fruits of the plants with application of Cu NPs presented the highest β -carotene content. The T0 under this condition had the lowest content. However, in refrigeration, the fruits of the plants of the two treatments that included the application of NaCl were those that had the highest β -carotene content. This indicates that the application of Cu NPs induces the formation and preservation of β -carotene when the fruits are stored at room temperature. This fact is very important, since β -carotene is the second most important carotenoid in the tomato and is an important antioxidant compound in human health [1]. As already mentioned, Cu NPs are believed to function as inducers of antioxidant compounds [11]. In addition, it has been demonstrated that the application of Cu NPs to the substrate induces the formation of lycopene in tomato [2].

Table 4. β -carotene content (milligrams per 100 g fresh weight) in tomato fruits at different storage times at room temperature ($24 \pm 1^\circ\text{C}$) and refrigeration ($4 \pm 1^\circ\text{C}$ and relative humidity at 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	1.17 ± 0.02 a	0.98 ± 0.01 a	1.74 ± 0.01 a	not done
	NaCl	0.99 ± 0.07 c	0.80 ± 0.01 c	1.56 ± 0.10 b	not done
	NPs Cu + NaCl	1.08 ± 0.02 b	0.95 ± 0.01 a	1.60 ± 0.04 b	not done
	T0	1.13 ± 0.02 ab	0.89 ± 0.01 b	1.33 ± 0.01 d	not done
Refrigeration	NPs Cu	not done	0.80 ± 0.01 c	1.17 ± 0.01 e	1.31 ± 0.01 c
	NaCl	not done	0.60 ± 0.01 d	1.32 ± 0.01 d	1.36 ± 0.01 a
	NPs Cu + NaCl	not done	0.59 ± 0.02 d	1.32 ± 0.04 d	1.28 ± 0.02 d
	T0	not done	0.83 ± 0.06 c	1.47 ± 0.02 c	1.34 ± 0.01 b

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L^{-1} . NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L^{-1} . T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

Finally, at 23 days of storage, the fruits of the plants with NaCl again showed the highest β -carotene content. This suggests that salinity stress induces the formation of this compound in fruits stored in refrigeration for more than 15 days.

The carotenoids are the most representative group of tetraterpenes [37]. β -carotene is the most important carotenoid for human diet and along with lycopene is the most important carotenoid in tomato fruits [1,3]. These compounds are biosynthesized from just two C_5 precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The biosynthesis of these universal terpene precursors via mevalonate [37]. Therefore, the production of these carotenoids is probably more related to oxidative stress responses caused by Cu NPs [10].

As with the previous compounds, there were also statistical differences between treatments at all evaluation times of vitamin C content (Table 5). At the time of harvest, the fruits of the plants with NaCl application only had the lowest content of vitamin C. The rest of the treatments were statistically equal. After eight days of storage at room temperature, the fruits of the plants with NaCl application had the lowest vitamin C content. Under this condition, the fruits of the plants with application of Cu NPs were significantly higher than those of the rest of the treatments. However, under refrigeration, the T0 showed the highest content of vitamin C, while the fruits with application of Cu NPs and NaCl had the lowest content.

Table 5. Vitamin C content (milligrams of ascorbic acid per 100 g fresh weight) in tomato fruits at different storage times at room temperature ($24 \pm 1^\circ\text{C}$) and refrigeration ($4 \pm 1^\circ\text{C}$ and relative humidity at 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	33.25 ± 0.27 ab	20.75 ± 0.40 b	26.12 ± 0.40 b	not done
	NaCl	32.89 ± 0.32 b	13.75 ± 0.41 f	19.11 ± 0.41 f	not done
	NPs Cu + NaCl	33.84 ± 0.31 a	14.28 ± 0.50 ef	19.65 ± 0.50 ef	not done
	T0	33.49 ± 0.52 ab	15.72 ± 0.31 d	21.08 ± 0.31 d	not done
Refrigeration	NPs Cu	not done	17.09 ± 0.36 c	22.45 ± 0.36 c	38.37 ± 0.23 a
	NaCl	not done	17.24 ± 0.47 c	22.60 ± 0.47 c	36.82 ± 0.32 ab
	NPs Cu + NaCl	not done	14.61 ± 0.34 e	19.98 ± 0.34 e	32.77 ± 0.37 b
	T0	not done	27.73 ± 0.44 a	33.10 ± 0.44 a	34.38 ± 4.93 ab

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L^{-1} . NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L^{-1} . T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

At 15 days of storage, the same treatment behavior was observed as in the previous evaluation. At room temperature, the fruits of the plants with NaCl application had the lowest vitamin C content, while the fruits of the plants with application of Cu NPs were significantly superior to the rest of the treatments. Under refrigeration, the T0 presented the highest content of vitamin C, and the fruits of the plants with application of Cu NPs and NaCl had the lowest content. At 23 days after storage in refrigeration, the fruits of plants with application of Cu NPs and NaCl had the lowest content of vitamin C, as it was in previous evaluations under these conditions. However, the fruits of the plants with application of Cu NPs had the highest vitamin C content. These results indicate that the application of Cu NPs increases the vitamin C content when the fruits are stored at room temperature. This may be derived from the induction of antioxidant compounds as mentioned above [11]. This is of great importance since humans must ingest vitamin C through rich sources such as fruits because the human body does not have the capacity to produce it [38]. Specifically in tomato fruits, vitamin C is one of the most important [1]. In the plants, vitamin C is converted from glucose following the pathway that involves the conversion of GDP-D-mannose to GDP-L-galactose catalyzed by a GDP-mannose-30,50-epimerase. L-galactose released from the nucleotide is the immediate precursor of L-galactono-1,4-lactone, which by action of a dehydrogenase is converted to L-ascorbic acid [39]. Thus the induction of this compound is more related to the antioxidant responses of plants [11].

In addition, it was also evident that salinity stress induces a significant decrease of vitamin C in tomato fruits stored at room temperature. This fact is probably due to the high stress generated by an oxidative burst leading to the reduction of antioxidants [9]. In this case, the vitamin C content was affected.

Regarding the antioxidant capacity by DPPH, only at 23 days of storage were there no differences between treatments (Table 6). Under conditions of room temperature, the fruits of plants with saline stress had the lowest values of antioxidant capacity during the three evaluations. The T0 showed the highest values at the time of harvest and at eight days of storage. At 15 days, the fruits of the plants with application of Cu NPs were those with higher antioxidant capacity, followed by the T0. These results suggest that the stress caused by the application of NaCl negatively affects the antioxidant DPPH ability, when the fruits are kept at room temperature (24 °C). This may be because the plants suffered a high stress by the constant application of NaCl, which triggered an oxidative burst [9], resulting in the reduction of antioxidant compounds.

Table 6. 2,2^t-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity (μM Trolox equivalents per gram of fresh weight) in tomato fruits at different storage times at room temperature (24 ± 1 °C) and refrigeration (4 ± 1 °C and relative humidity at 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	2.09 \pm 0.02 a	1.93 \pm 0.01 f	2.09 \pm 0.01 a	not done
	NaCl	2.07 \pm 0.01 b	1.75 \pm 0.01 h	1.85 \pm 0.02 e	not done
	NPs Cu + NaCl	2.09 \pm 0.02 a	1.87 \pm 0.02 g	1.63 \pm 0.02 f	not done
	T0	2.09 \pm 0.01 a	2.09 \pm 0.01 b	2.01 \pm 0.01 b	not done
Refrigeration	NPs Cu	not done	2.03 \pm 0.01 d	1.92 \pm 0.01 c	2.14 \pm 0.03 a
	NaCl	not done	1.98 \pm 0.01 e	1.91 \pm 0.01 cd	2.12 \pm 0.02 a
	NPs Cu + NaCl	not done	2.05 \pm 0.01 c	1.89 \pm 0.01 d	2.11 \pm 0.02 a
	T0	not done	2.12 \pm 0.01 a	2.04 \pm 0.01 b	2.13 \pm 0.01 a

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L⁻¹. NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L⁻¹. T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

Under refrigeration conditions, the T0 had the highest antioxidant capacity at 8 and 15 days of storage. At eight days, the fruits of the plants with application of NaCl presented the lowest value.

At 15 days, the lowest antioxidant capacity was observed in the fruits of the plants with application of Cu NPs and NaCl.

On the other hand, in the antioxidant capacity by ABTS differences between treatments were presented in all evaluations. At the time of harvest, the T0 had the highest antioxidant capacity. In this case, the fruits of the plants of the two treatments that included the application of NaCl had lower antioxidant capacity.

At eight days of storage at room temperature, the fruits of plants with saline stress had the lowest values of antioxidant capacity. In this case, the fruits of the plants with application of Cu NPs presented the highest value of antioxidant capacity. Under refrigeration conditions, the opposite was observed, this treatment had the lowest antioxidant capacity. The T0 in this case presented the highest value of antioxidant capacity.

At 15 days, storage at room temperature, the results were consistent with that observed in previous evaluations. The fruits of the plants with saline stress had the lowest values of antioxidant capacity. In this condition, the fruits of the T0 plants obtained the best results, followed by the fruits of the plants with application of Cu NPs. However, under refrigeration, the fruits of the plants with NaCl presented significantly higher antioxidant capacity, while the fruits of the T0 plants present the lowest value. The same was observed after 23 days of storage under refrigeration.

These results suggest that when the fruits are maintained at room temperature (24 °C), the stress caused by the application of NaCl negatively affects the antioxidant capacity evaluated by ABTS. Under this same condition, the application of Cu NPs can increase the antioxidant capacity at least at some point in storage. However, when fruits are stored in refrigeration (4 °C), the opposite is observed. Application of NaCl at 15 and 23 days of storage significantly increases ABTS antioxidant capacity (50–150%) compared to T0. Since the ABTS technique measures the hydrophilic compounds [40], it is probable that the refrigeration condition modified the behavior of these antioxidants present in the fruits.

The behavior observed in antioxidant activity was different for both DPPH and ABTS methods, and no correlation between these results was observed. This is due the differences between both methods: the ABTS technique measures the hydrophilic compounds [40], while the DPPH technique can be used to examine both hydrophilic and lipophilic antioxidants [41]. There are also differences in reaction times, and it is specific for each type of antioxidant [28,42], which results in differences in the quantification of antioxidant activity as observed here (Tables 6 and 7). For this reason, a single method is not suitable for measuring all antioxidants in plants, and there is no shortcut approach to determine antioxidant activity as mentioned by [41].

Table 7. 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) antioxidant capacity (μM Trolox equivalents per gram of fresh weight) in tomato fruits at different storage times at room temperature (24 ± 1 °C) and refrigeration (4 ± 1 °C and relative humidity at 80%).

Storage	Treatment	0 ds	8 ds	15 ds	23 ds
Room temperature	NPs Cu	2.33 \pm 0.03 b	2.93 \pm 0.04 a	2.34 \pm 0.08 c	not done
	NaCl	2.17 \pm 0.03 c	2.34 \pm 0.01 e	2.02 \pm 0.06 d	not done
	NPs Cu + NaCl	2.21 \pm 0.03 c	2.04 \pm 0.04 f	2.08 \pm 0.04 d	not done
	T0	2.53 \pm 0.03 a	2.79 \pm 0.02 b	2.55 \pm 0.11 b	not done
Refrigeration	NPs Cu	not done	2.40 \pm 0.07 e	2.34 \pm 0.04 c	2.86 \pm 0.05 b
	NaCl	not done	2.65 \pm 0.04 c	3.84 \pm 0.09 a	3.29 \pm 0.06 a
	NPs Cu + NaCl	not done	2.53 \pm 0.05 d	1.57 \pm 0.05 e	2.67 \pm 0.05 c
	T0	not done	2.76 \pm 0.04 b	1.52 \pm 0.02 e	2.26 \pm 0.06 d

ds: days of storage. NPs Cu: plants without stress and with application Cu nanoparticles to 250 mg L⁻¹. NaCl: plants with stress by NaCl. NPs Cu + NaCl: plants with stress by NaCl and with application Cu nanoparticles at 250 mg L⁻¹. T0: absolute control without application of Cu nanoparticles and without stress by NaCl. Each value is expressed as mean \pm standard deviation ($n = 3$). Different letters by column indicate statistical differences between treatments according to Fisher's least significant difference test ($p \leq 0.05$).

On the other hand, a correlation between antioxidant activity and bioactive compounds exist only for some specific antioxidants. For example, in tomato fruits, antioxidant activity DPPH increased when phenolic and lycopene contents increased, but flavonoid and β -carotene decreased [34]. In tomato fruits under drought stress, the antioxidant activity ABTS, lycopene, total phenolics, and flavonoids increased, but the increase between this compounds was not in the same magnitude [3]. In the results observed here, a correlation between the bioactive compounds evaluated and antioxidant activity is unclear. However, it should be consider that antioxidant activity is due to action of all antioxidants in the fruits [41].

Moreover, the changes observed in all variables evaluated during post-harvest should be due to different factors that affect them. It is well known that the firmness of a tomato continuously decreases during storage [2] due to ethylene synthesis and respiration [43]. This affect diminishes membrane integrity, which could cause a decompartmentalization of texture-related enzymes and their substrates, leading to a rise in fluids and solute exchanges as well as an increase in enzymatic activity [43], which in turn affect the bioactive compounds and antioxidant activity.

4. Conclusions

The application of copper nanoparticles and NaCl during the development of tomato plants modifies the accumulation and degradation patterns of bioactive compounds in the postharvest life of the fruits.

The application of copper nanoparticles in general has a positive effect on the accumulation of bioactive compounds, mainly total phenols, β -carotene and vitamin C. This response is related to antioxidant responses of plants. On the contrary, the application of NaCl during the development of tomato plants causes a decrease of the bioactive compounds and antioxidant capacity in tomato fruits. This is due to high stress by the constant application of NaCl, which triggers an oxidative burst resulting in the reduction of these compounds.

The negative effect of the application of NaCl on fruits can be reduced with the application of copper nanoparticles via foliar, especially when the fruits are stored under conditions of room temperature of around 24 °C.

The application of copper nanoparticles may be a technique to increase and maintain the content of bioactive compounds in tomato fruits. In addition, it can be an effective alternative to diminish the negative effects on bioactive compounds content caused by the saline stress.

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CONCLUSIÓN GENERAL

La aplicación foliar de NPs Cu induce la acumulación de compuestos bioactivos como la vitamina C y licopeno en frutos de tomate.

Así mismo, modifica positivamente la actividad de las principales enzimas encargadas de eliminar las especies reactivas de oxígeno.

Además, puede ser una alternativa efectiva para disminuir los efectos negativos sobre a acumulación y el contenido de compuestos antioxidantes causados por el estrés salino.

Por lo tanto, con la aplicación de NPs Cu se generan frutos de tomate de mejor calidad para la dieta humana debido a la acumulación de compuestos bioactivos.

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