Universidad Autónoma de Baja California.

Instituto de Ciencias Agrícolas.

Respuesta fisiológica, bioquímica y molecular de nanocompuestos de sílice con oligoméros de quitosano y cobre en el control de la roya del café en *Coffea arabica.*

T E S I S

QUE COMO REQUISITO PARCIAL

PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS AGROPECUARIAS

PRESENTA

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Mexicali, Baja California 22 de octubre del 2025.

La presente tesis **“*Respuesta fisiológica, bioquímica y molecular de nanocompuestos de sílice con oligoméros de quitosano y cobre en el control de la roya del café en Coffea arabica*”** realizada por el **C. Alexis Alejandro Salazar Navarro**, dirigida por el **Dr. Daniel González Mendoza** y el **Dr. Víctor Manuel Ruíz Valdiviezo**, ha sido evaluada y aprobada por el Comité Particular abajo indicado, como requisito parcial para obtener el grado de:

Doctor en Ciencias Agropecuarias

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Agradecimientos.

Este trabajo de tesis, realizado en el Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California, está dedicado con profunda gratitud a todas las personas y organizaciones que, de forma directa o indirecta, fueron fundamentales para su desarrollo.

A mi director de tesis, el Dr. Daniel González Mendoza, y mi codirector, el Dr. Víctor Manuel Ruíz Valdiviezo, por su calidad humana, paciencia y compromiso. Gracias por acompañarme a lo largo de este camino, brindándome una guía constante tanto en el ámbito académico como personal.

A los miembros de mi comité de tesis, la Dra. Olivia Tzintzun Camacho, el Dr. José Gregorio Joya Dávila y el Dr. Ulin Antobelli Basilio Cortés, por sus valiosos consejos y por extender su orientación más allá de lo académico, guiándome también en mi desarrollo profesional.

Agradezco al Instituto de Ciencias Agrícolas (UABC), al Instituto Tecnológico Nacional de México, Campus Tuxtla Gutiérrez, y al Instituto Hondureño del Café (IHCAFÉ), por abrirme sus puertas, por permitirme colaborar con personas excepcionales, y por brindarme el respaldo necesario para llevar a cabo este proyecto. A la SECIHTI, gracias por su apoyo y por incentivar a las y los jóvenes a continuar su formación de posgrado.

A mi familia, por su amor incondicional, por confiar siempre en mí y por acompañarme en cada etapa de mi vida. A mi padre, por enseñarme a no rendirme; a mi madre, por su paciencia y constante aliento; a mis hermanos, por sus consejos; y a mi sobrina Romina, por llenar mis días de alegría.

A Carlos, por acompañarme desde la licenciatura, por su escucha atenta, su apoyo inquebrantable y por compartir conmigo cada proyecto de vida, impulsándome siempre a descubrir nuevos horizontes. A mis hijos de cuatro patas, Elio, Percy, Lúa, Mila y Mila, por acompañarme con su amor incondicional y alegrar mis días incluso en los momentos más difíciles.

A mis amigas del TecNM, Abril y Andy, por hacer más amenas y enriquecedoras mis estancias de investigación. A mis amigos de siempre, André, Sofía, Rafael, Eduardo y Michelle, por su apoyo constante, sus consejos y su valiosa compañía a lo largo de los años. A los equipos de mis proyectos: Cultura Barista, Café Contigo y Café Adxé, gracias por acompañarme en este camino, por su apoyo diario y por permitirme construir sueños que caminan junto a mi formación académica.

A cada uno de ustedes, gracias por su presencia y apoyo incondicional. Este logro también es suyo. Mi más sincero agradecimiento, hoy y siempre.

TABLA DE CONTENIDO

Resumen

La roya de la hoja del café, causada por *Hemileia vastatrix*, representa una de las principales amenazas para la productividad y calidad de *Coffea arabica* en países productores. Ante las limitaciones de métodos convencionales como el uso de fungicidas cúpricos y variedades resistentes con eficacia limitada, esta tesis exploró alternativas biotecnológicas basadas en nanopartículas de sílice (SiNPs), solas o funcionalizadas con oligómeros de quitosano (COS) e iones cúpricos (Cu^{2+}), como elicitores foliares en *C. arabica* var. *Bourbon*.

Los COS se sintetizaron mediante hidrólisis química, mecánica y oxidativa, y se obtuvieron nanocompuestos funcionalizados (SiNPs-COS y SiNPs-COSCu) por el método Sol-Gel. Su aplicación foliar indujo respuestas bioquímicas evidenciadas por un aumento en la actividad de enzimas relacionadas con defensa (PAL, β-1,3-glucanasa, quitinasa, catalasa y peroxidasa), así como estabilidad en parámetros fotosintéticos, sin efectos fitotóxicos.

Además, se evaluó la expresión génica de seis marcadores de defensa (*PAL*, *PR1*, *PR5*, *CaNDR1*, *NBS-LRR* y *β-1,3-glucanasa*), observándose una inducción significativa, especialmente con SiNPs-COS y SiNPs-COSCu. Estos resultados destacan su papel en la activación de rutas asociadas con la resistencia sistémica adquirida (SAR) y la respuesta hipersensible (HR).

Finalmente, en ensayos con discos foliares tratados e inoculados con urediniosporas de *H. vastatrix*, se observó un efecto preventivo con menor germinación de esporas y daño celular reducido, medido por fugas electrolíticas y tinciones histoquímicas.

Estos resultados respaldan el uso de nanocompuestos funcionalizados como agentes preventivos en el manejo sostenible de enfermedades en café, al inducir tempranamente respuestas bioquímicas y moleculares de defensa.

Palabras clave: Biotróficos, fitopatógenos, nanopartículas, roya de la hoja del café.

Abstract

Coffee leaf rust (CLR), caused by *Hemileia vastatrix*, is one of the most critical threats to the productivity and quality of *Coffea arabica* in producing countries. Due to the limited effectiveness of conventional methods—such as copper-based fungicides and resistant cultivars—this thesis explores biotechnological alternatives using silica nanoparticles (SiNPs), either unmodified or functionalized with chitosan oligosaccharides (COS) and copper ions (Cu^{2+}), as foliar elicitors in *C. arabica* var. *Bourbon*.

COS were synthesized through chemical, mechanical, and oxidative hydrolysis, and functionalized nanocomposites (SiNPs-COS and SiNPs-COSCu) were obtained via the Sol-Gel method. Foliar applications induced biochemical responses, including increased activity of defense-related enzymes (PAL, β-1,3-glucanase, chitinase, catalase, and peroxidase), along with stable photosynthetic parameters and no signs of phytotoxicity.

The molecular response was also assessed by quantifying the expression of six defense-related genes (*PAL, PR1, PR5, CaNDR1, NBS-LRR*, and *β-1,3-glucanase*). Treatments with SiNPs-COS and SiNPs-COSCu significantly upregulated genes associated with systemic acquired resistance (SAR) and hypersensitive response (HR), highlighting their elicitor potential.

Additionally, foliar disc assays were developed using leaves treated with the nanocomposites and inoculated with *H. vastatrix* urediniospores. A preventive effect was observed, including reduced spore germination and lower cellular damage, confirmed by electrolyte leakage measurements and histochemical staining.

These results support the application of functionalized nanomaterials—especially SiNPs-COS and SiNPs-COSCu—as early inducers of molecular and biochemical defenses in *C. arabica*, positioning them as promising tools for the sustainable and preventive management of coffee leaf rust and other biotic stress.

Keywords: Biotrophics, phytopathogens, nanoparticles, coffee leaf rust.

CAPÍTULO 1. INTRODUCCIÓN

Estructura de la tesis

Esta tesis se encuentra estructurada por capítulos derivados de productos de investigación científica, incluyendo artículos publicados, en proceso editorial o en preparación. Cada capítulo aborda distintos aspectos del proyecto, desde el contexto fitopatológico hasta la evaluación funcional de los nanocompuestos, conforme a los objetivos planteados.

El primer capítulo busca ofrecer un acertamiento al tema de investigación, mientras que los capítulos subsecuentes se establecen las bases científicas del proyecto. Durante el segundo capítulo se genera una introducción al área de nanotecnología, mencionando las principales rutas de síntesis de nanomateriales, así como los posibles materiales precursores de nanopartículas y sus posibles modificaciones enfocadas en el cumplimiento de una función específica, a lo que se denomina funcionalización. Además, en este capítulo se abordan técnicas de caracterización y se comienzan a explorar diversas aplicaciones de nanopartículas en la agricultura.

A partir del tercer capítulo se define la problemática central de la investigación, la roya de la hoja del café causada por *Hemileia vastatrix*. Durante este capítulo se explica el ciclo reproductivo y la patogenia de *H. vastatrix*, así como se establecen las bases de la coevolución que existe entre especies del género *Coffea* y *H. vastatrix*. Además, se explican los mecanismos bioquímicos y moleculares que definen las relaciones planta-patógeno que generan respuestas de compatibilidad e incompatibilidad, fundamentales para el entendimiento de los mecanismos de acción de las nanopartículas diseñadas durante el presente proyecto de investigación.

En los siguientes capítulos se abordan diversos resultados del proyecto. Por ejemplo, en el cuarto capítulo se describe el proceso de síntesis y funcionalización de nanopartículas de silicio con oligoméros de quitosano y cationes de cobre (SiNPs-COS@Cu), el objetivo de este capítulo se baso en la obtención de las nanopartículas y comprobar su bioactividad, por lo que se presentan resultados de caracterización de nanomateriales junto antibiogramas de bacterias patogenas como *E. coli* y *S. aureus*. En el quinto y sexto capítulo se comienza a explorar la respuesta bioquímica de plantas jóvenes de café (*Coffea arabica* var. Bourbon) ante la aplicación foliar de oligoméros de quitosano y nanopartículas de silicio, respectivamente. Esto, con la finalidad de estudiar el efecto de los precursores del nanomaterial compuesto (SiNPs-COS@Cu) en el espécimen de interés. En ambos capítulos se busca entender los mecanismos bioquímicos que los precursores pueden encender en la planta, y si estas respuestas pueden relacionarse con una posible fitotoxicidad o con activación de mecanismos de defensa. A partir de estos resultados, se logró establecer rangos de concentración de los precursores que no representen riesgo para la planta pero que le puedan ayudar a superar la invasión de uredinosoras de *H. vastatrix.*

Para el sexto capítulo, se estudia la expresión de genes relacionados con mecanismos de defensa horizontal y vertical en plantas jóvenes de *Coffea arabica* var. Bourbon elicitadas con los nanocompuestos (SiNPs-COS@Cu). Estos resultados ayudan a establecer las bases moleculares de los mecanismos de acción de los nanotratamientos para sus futuras aplicaciones *in vivo* e *in planta*. Los resultados derivados del sexto capítulo son fundamentales para establecer las conclusiones del proyecto y perspectivas futuras, buscando que la presente investigación pueda trascender más allá de una tesis doctoral.

Introducción

La roya del café, causada por *Hemileia vastatrix*, continúa siendo el principal limitante fitosanitario de *Coffea arabica* en América Latina. Este patógeno ha demostrado una alta capacidad de adaptación, lo que ha provocado brotes epidémicos incluso en variedades consideradas previamente resistentes (Barquero, 2013; Calzada Rovirosa, 2017; Cárdenas, 2007; CEDRASSA, 2018; CEDRSSA, 2019; Romero y Camili, 2019). México, uno de los principales productores de café de la región, ha sido severamente afectado por la presencia de *H. vastatrix* desde el 2021, registrando la mayor cantidad de pérdidas en el 2016, alcanzando el 50% de la producción nacional, generando consecuencias tanto económicas como sociales (CEDRASSA, 2018; CEDRSSA, 2019; Romero y Camili, 2019).

Los métodos de control convencionales, basados en el uso intensivo de fungicidas cúpricos y la renovación con variedades resistentes, han mostrado limitaciones en cuanto a sostenibilidad, eficacia a largo plazo y aceptación por parte de los productores (Barquero, 2013; Joaquín Durán Mora et al., 2017). Esto ha impulsado la búsqueda de alternativas más sostenibles y biotecnológicas que permitan inducir mecanismos de defensa en la planta y reducir la dependencia de agroquímicos.

Dentro de este contexto, la nanotecnología agrícola ha emergido como una herramienta prometedora. En particular, las nanopartículas de sílice (SiNPs), funcionalizadas con compuestos bioactivos como oligómeros de quitosano (COS) e iones cúpricos, han mostrado potencial como elicitores capaces de activar respuestas bioquímicas y moleculares en las plantas sin efectos fitotóxicos (Salazar-Navarro et al., 2025, 2023). No obstante, su aplicación en conjunto como un nanosistema con 3 capas de funcionalización no se ha explorado en cultivos tropicales de alto valor como el café.

Justificación

La roya de la hoja del café (CLR), causada por las uredinosporas de *Hemileia vastatrix*, es una enfermedad devastadora que afecta principalmente a especies del género *Coffea*. Este hongo biotrófico obligado mantiene una estrecha relación coevolutiva con *Coffea arabica*, lo que ha llevado al desarrollo de variedades con mecanismos de defensa más eficientes para reconocer e inhibir las etapas de infección del patógeno.

Sin embargo, la eficacia del mejoramiento genético orientado a inducir resistencia en *C. arabica* se ve amenazada por la aparición constante de nuevas razas de *H. vastatrix*, capaces de evadir los sistemas de detección de las plantas y establecer una relación compatible. Esta situación ha debilitado el enfoque basado en la resistencia vertical, como se observa en el caso del Híbrido de Timor, cuya resistencia ha sido superada por nuevas variantes del patógeno.

Paralelamente, el uso extensivo de pesticidas convencionales ha generado preocupación debido a sus posibles efectos tóxicos y su bioacumulación en los ecosistemas cafetaleros. Ante esta problemática, el sector requiere nuevas estrategias sostenibles que no comprometan la salud ambiental ni la productividad a largo plazo.

Entre las alternativas emergentes, la nanotecnología y la biotecnología ofrecen herramientas prometedoras. Destaca el uso de nanopartículas capaces de inducir mecanismos de defensa no específicos, conocidos como resistencia horizontal, que pueden activarse mediante moléculas exógenas denominadas elicitores. A diferencia de la resistencia vertical, esta estrategia no promueve la aparición de nuevas razas del patógeno y puede ser aplicada a variedades tradicionales sin necesidad de reemplazo.

En este contexto, la presente tesis propone evaluar, desde un enfoque integral, el efecto de nanomateriales funcionalizados y sus precursores (SiNPs, SiNPs-COS, SiNPs-COSCu) sobre parámetros fisiológicos, bioquímicos y moleculares en plantas de *Coffea arabica* var. Borbón.

Hipótesis general

La aplicación foliar de nanopartículas de sílice funcionalizadas con oligómeros de quitosano e iones cúpricos induce mecanismos moleculares de defensa que activan rutas bioquímicas que inhiben la interacción del hongo causante de la roya del café (*Hemileia vastatrix*) en plantas de *Coffea arabica*.

Objetivo general

Evaluar el efecto de nanopartículas de sílice funcionalizadas con COS y Cu^{2+} sobre respuestas bioquímicas, fisiológicas y moleculares en plantas de *Coffea arabica* var. *Bourbon* bajo condiciones de invernadero, con énfasis en su potencial como agentes elicitores frente a *Hemileia vastatrix*.

Objetivos específicos

1. Sintetizar y caracterizar físico-químicamente nanopartículas de sílice funcionalizadas con COS y Cu^{2+}.
2. Evaluar la respuesta bioquímica de plantas de *C. arabica* tratadas con los nanocompuestos, mediante análisis enzimáticos y fisiológicos.
3. Analizar la expresión de genes asociados a mecanismos de defensa inducida en plantas tratadas.
4. Estimar el efecto preventivo de los nanocompuestos mediante ensayos con discos foliares inoculados con urediniosporas de *H. vastatrix*

Diagrama metodológico

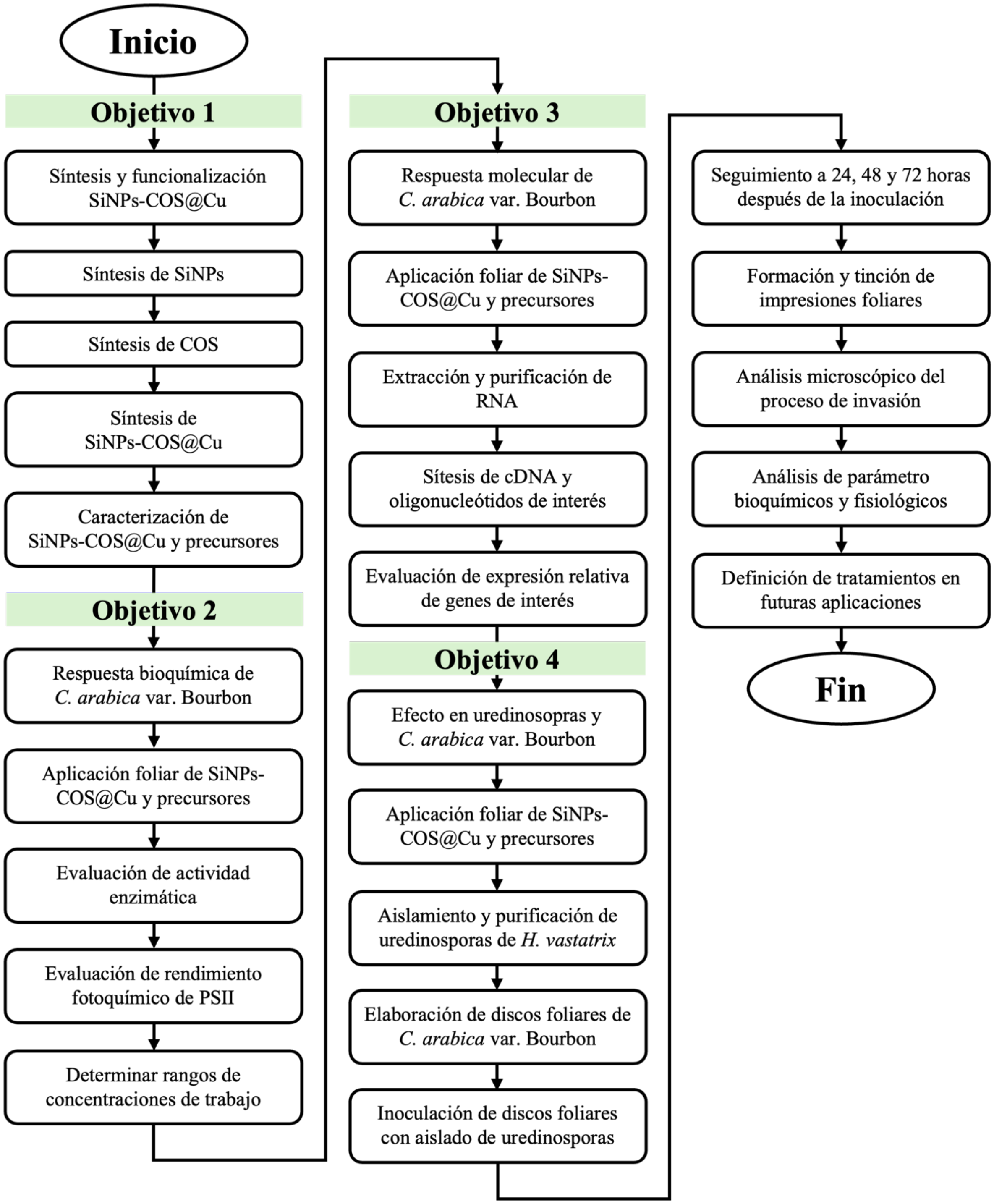

Figura 1. Diagrama metodológico general de las etapas realizadas para cada objetivo específico.

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CAPÍTULO 2. SÍNTESIS, CARACTERIZACIÓN Y APLICACIONES DE NANOPARTÍCULAS EN LA AGRICULTURA: UNA REVISIÓN TÉCNICA E INTRODUCTORIA

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Capítulo enviado para su publicación en la obra académica: "Los sistemas alimentarios en regiones tropicales latinoamericanas", editado y publicado por La Escuela de Sistemas Alimentarios (SiAL) de la Universidad Autónoma de Chiapas (UNACH). En proceso editorial (UNACH, 2025).

RESUMEN

El uso intensivo de compuestos químicos en la agricultura, como fertilizantes, pesticidas y promotores de crecimiento, ha provocado efectos negativos sobre el suelo y los cuerpos de agua subterráneos. En este contexto, los nanomateriales emergen como una alternativa viable y sostenible, gracias a sus propiedades fisicoquímicas únicas que permiten una mayor eficiencia en dosis reducidas. La síntesis de nanopartículas puede realizarse mediante rutas químicas, físicas o biológicas, y su diseño depende del objetivo específico de aplicación. Estas partículas pueden ser de naturaleza orgánica, inorgánica, basada en carbono o combinaciones híbridas, lo que permite una amplia gama de aplicaciones agrícolas. Además, es posible funcionalizar los nanomateriales mediante encapsulación o recubrimientos para mejorar su estabilidad, liberación dirigida o interacción con organismos. Este capítulo presenta una revisión de los métodos de síntesis, caracterización y aplicaciones potenciales de nanopartículas en la agricultura, destacando su uso como agentes de control de fitopatógenos, promotores del crecimiento vegetal, elicitores de respuestas de defensa y sistemas de fortificación de cultivos. Se discuten también técnicas clave de caracterización, fundamentales para comprender el impacto de las propiedades fisicoquímicas en su desempeño agrícola.

Palabras clave: Elicitores de defensa, control de fitopatógenos, nanomateriales, funcionalización, promotores de crecimiento.

SUMMARY

The intensive use of chemical compounds in agriculture, such as fertilizers, pesticides, and growth promoters, has led to environmental degradation of soils and groundwater. In this context, nanomaterials have emerged as a viable and sustainable alternative due to their unique physicochemical properties, which enable greater efficiency at lower doses. Nanoparticles can be synthesized through chemical, physical, or biological routes, and their design depends on the specific agricultural application. Their composition may be organic, inorganic, carbon-based, or hybrid, allowing for broad functionality. Moreover, nanomaterials can be functionalized through encapsulation or coatings to enhance their stability, targeted delivery, or biological interactions. This chapter presents a comprehensive review of synthesis methods, characterization techniques, and potential applications of nanoparticles in agriculture. Particular emphasis is placed on their use as agents for phytopathogen control, plant growth promotion, elicitation of defense responses, and crop fortification. Key characterization approaches are also discussed as essential tools for understanding the impact of nanoparticle physicochemical properties on agricultural performance.

Keywords: Defense elicitors, phytopathogen control, nanomaterials, functionalization, growth promoters.

INTRODUCCIÓN

NANOTECNOLOGÍA Y NANOPARTÍCULAS: DEFINICIONES BÁSICAS

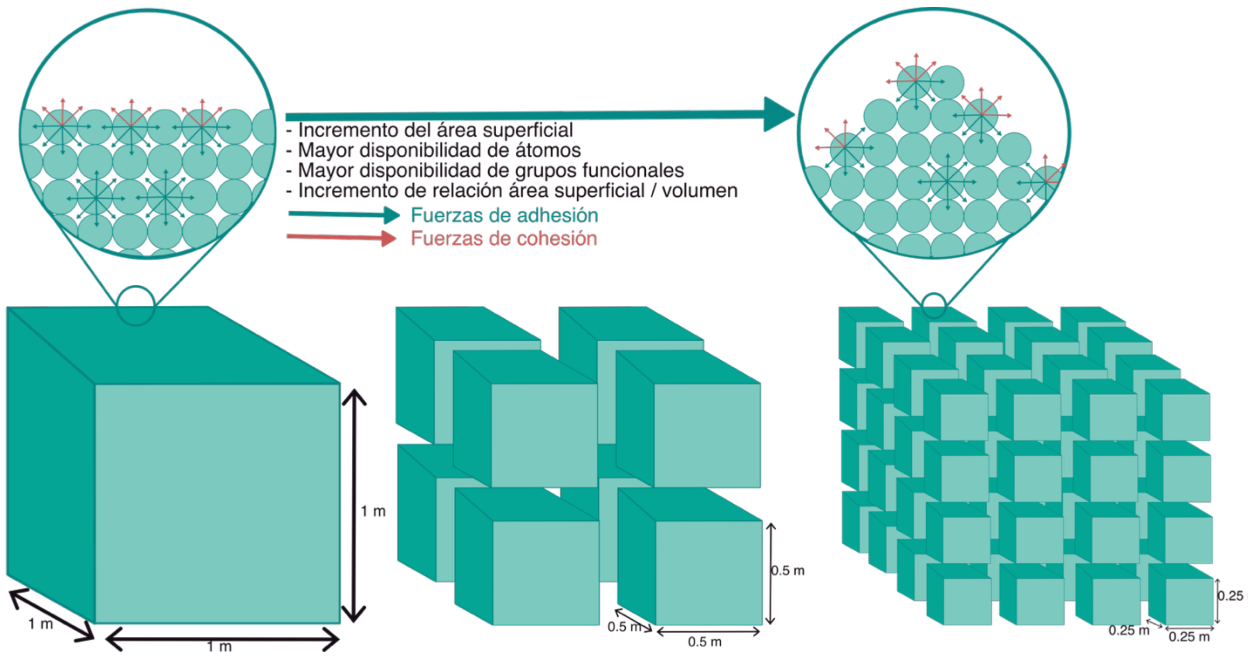
Para comenzar a hablar de nanopartículas (NPs) y sus aplicaciones, es importante iniciar con las definiciones básicas de nanotecnología, mismas que gobiernan su desempeño, funcionalidad y propiedades fisicoquímicas. El término de nanotecnología proviene del griego "*nano*" que en un origen tomaba la definición de "enano", hoy en día, "nano" se relaciona con el prefijo (10^{-9}) y su significado para hablar de una milmillonésima parte de algo (Navarro Espinoza et al., 2021; Thakur and Thakur, 2022). Por lo que cuando se habla de nanotecnología, se puede mencionar aquellas tecnologías, técnicas y herramientas que permiten diseñar, manipular y trabajar con los materiales del orden nanométrico (Saritha et al., 2022). La nanotecnología puede definirse como una ciencia multidisciplinaria basada en ciencias básicas como la biología, física, química y geología pero que puede aplicarse en diversas áreas de la ciencia como medicina, cosmética, farmacología, ciencia de los materiales, ciencias agrícolas, electrónica, entre otras (Prasad et al., 2022; Thakur and Thakur, 2022).

Por lo que se puede definir el término de nanopartículas como partículas dentro del rango nanométrico o que sus mediciones se encuentran alrededor de los nanómetros (nm, 10^{-9} m), aunque esto es cierto, hablando de funcionalidad, únicamente se consideran como nanopartículas aquellas que en cualquiera de sus ejes (X, Y, Z), presenten una medición de 1 a 100 nm (Salazar-Navarro et al., 2024; Thakur and Thakur, 2022). Esto ocurre ya que dentro del rango de 1 a 100 nm algunas propiedades fisicoquímicas se potencializan o mejoran su desempeño debido al incremento de la relación que existe entre su área superficial y volumen (Figura 1), lo que incrementa la disponibilidad de sus átomos por encontrarse en la superficie y reaccionar con otros elementos o materiales (Findik, 2021). Además, su tamaño nanométrico favorece su transporte celular ya que dentro del rango de 1 a 100 nm se mejora su comportamiento coloidal (Ahmad et al., 2022; Beach et al., 2024).

La nanotecnología ha ganado popularidad últimamente en la agricultura, por ejemplo, se ha propuesto como una emergente alternativa para el control de plagas y enfermedades basado en la aplicación de fungicidas a base de nanopartículas, por ejemplo, en mejorar la eficiencia del producto, esto debido a que las nanopartículas del rango de 1-100 nm suelen comportarse como “moléculas grandes” en lugar de materiales en forma de “bulto” (Figura 1), lo que les permite tener una mayor interacción química con sus objetivos (Elsharkawy et al., 2022; Sabir et al., 2022). Por ejemplo, se han reportado la aplicación de nanopartículas de selenio en el control de hongos como *Aspergillus niger* y *Candida albicans*, así como la actividad antifúngica de nanopartículas de zinc contra *Aspergillus fumigatus*, a su vez, se han aplicado las nanopartículas de cobre en el control de *Tribolium castaneum*, la actividad antimicrobiana de nanopartículas de plata y la capacidad de actuar como vehículos de nanopartículas de sílice, titanio y quitosano (El-Saadony et al., 2020; Jaiswal et al., 2012; Kazempour et al., 2013; Neeran, 2017; Ramezani et al., 2019; Zulfiqar et al., 2019). Adicionalmente, las nanopartículas obtenidas, se pueden funcionalizar mediante modificaciones superficiales, por ejemplo, se ha reportado la modificación de SiNPs con oligómeros de quitosano

(COS) para el control de bacterias como *Escherichia coli* (*E. coli*) y *Staphylococcus aureus* (*S. aureus*) (Salazar-Navarro et al., 2023).

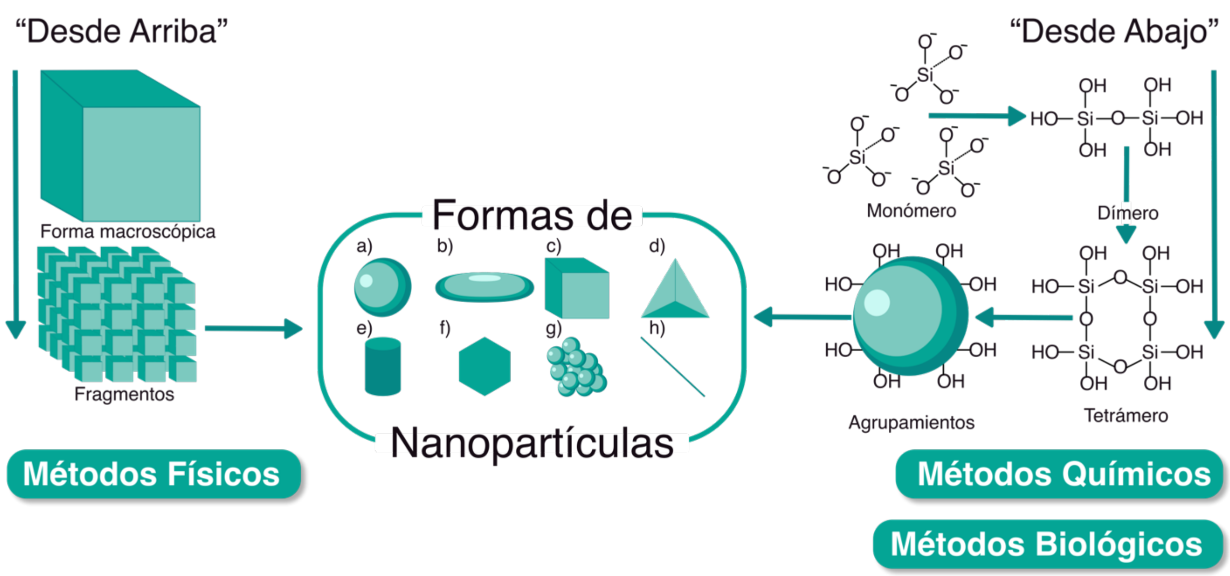
Figura 1. Comparación de los cambios fisicoquímicos y comportamiento de un material en forma nanoparticulada y en su forma macroscópica o de "bulto" (Kumari et al., 2023; Lu et al., 2020).



RUTAS Y METODOLOGÍAS DE SÍNTESIS

Las principales rutas de síntesis de nanopartículas suelen dividirse en diferentes segmentos, dependiendo del origen del material precursor y dependiendo del tipo de fuerza aplicada para la síntesis. Por ejemplo, dependiendo del origen del precursor, las rutas de síntesis pueden dividirse como "Bottom-up" y "Top-down" que se podrían traducir como "desde abajo" y "desde arriba" ya que hablan del origen del material precursor (Figura 2) (Kumari et al., 2023; Salazar-Navarro et al., 2024; Szczyglewska et al., 2023). Cuando este, se obtiene desde un precursor químico como una sal o un compuesto granulado, se aprovechan los átomos del elemento de interés para “construir” una partícula tridimensional que idealmente se mantenga dentro del rango de 1-100 nm, se habla de las rutas “desde abajo” o “bottom-up”. Por ejemplo, en la Figura 2 se representa la construcción nanopartículas de silicio (SiNPs) a partir de ácido silícico generado como monómero para su posterior polimerización controlada (Salazar-Navarro and Salas-Valdez, 2022). En cambio, cuando se parte de un material macroscópico como una lámina, un electrodo o incluso una piedra que contenga los elementos de interés y son sometidos a procesos de degradación para obtener partículas del orden nanométrico, se habla de las rutas “desde arriba” o “top-down”. A su vez, las rutas de síntesis pueden dividirse en base a la técnica empleada, por ejemplo, si se siguió una síntesis química, mecánica o biológica (Abid et al., 2022; Baig et al., 2021).

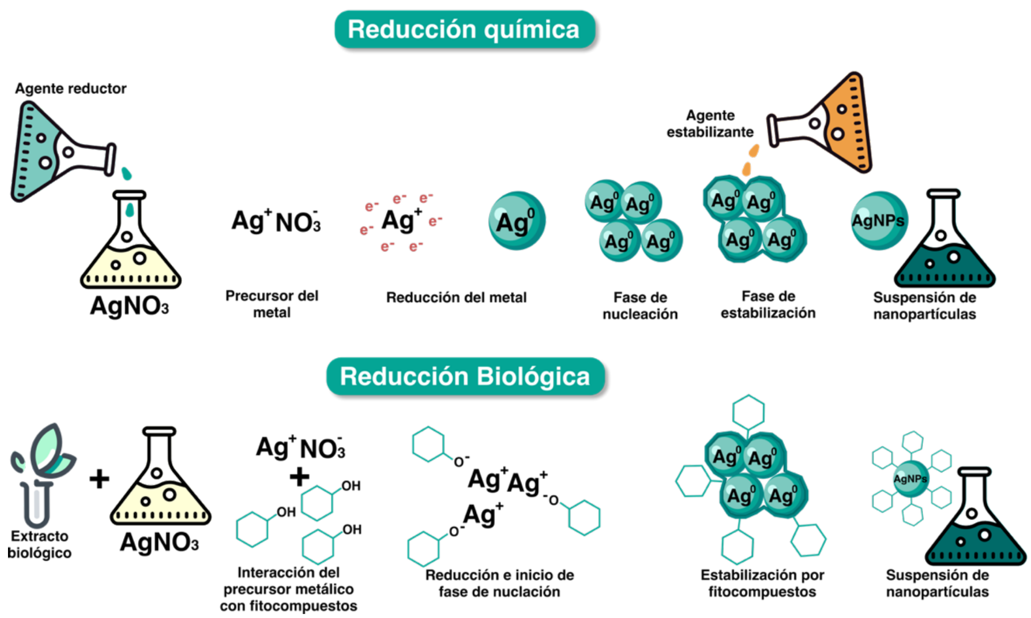
Figura 2. Principales rutas de síntesis de nanopartículas, partiendo “desde arriba” con materiales macroscópicos o “desde abajo” con precursores químicos; para la obtención de nanopartículas de forma a) esférica, b) ovalada, c) cubica, d) piramidal, e) cilíndricas, f) hexagonales, g) aglomeradas, h) aguja, entre otras (Hamida et al., 2020; Kumari et al., 2023; Salazar-Navarro and Salas-Valdez, 2022).



La síntesis química suele abarcar las metodologías Sol-Gel, a partir de la formación de “Soles (Sol)” que posteriormente tenderán a gelificar “Geles (Gel)” para formar estructuras sólidas, uno de los puntos clave en esta metodología es controlar la velocidad de gelación, un parámetro determinante para definir el tamaño de las nanopartículas y su homogeneidad. Mismo que suele controlarse modificando parámetro como materiales precursores, concentración de electrolitos, pH, temperatura, velocidad de agitación, uso de solventes orgánicos y su velocidad de adición. La metodología Sol-Gel suele aplicarse mayormente a la síntesis de SiNPs pero también puede aplicarse para obtener TiNPs y ZnNPs (Brinker and Scherer, 1990; Gonçalves, 2018; Jeffrey Brinker, 2005).

Otra popular ruta de síntesis química es la reducción química de metales (Figura 3), principalmente empleada para la obtención de AgNPs, CuNPs, ZnNPs, AuNPs y SeNPs. Durante esta ruta, se parte de sales precursoras del metal de interés, por ejemplo, nitrato de plata ($AgNO_3$), sulfato de cobre ($CuSO_4$), nitrato de zinc ($ZnNO_3$), por lo general disueltas en agua destilada, a las que se les agrega un agente reductor en presencia de un agente estabilizante, esto con la finalizad de reducir los metales disueltos en agua destilada para su posterior agregación y estabilización o recubrimiento con el agente estabilizante (Guilger-Casagrande and Lima, 2019; Hamida et al., 2020; Kumari et al., 2023; Szczyglewska et al., 2023). Los puntos críticos en esta metodología es encontrar sales precursoras solubles en el solvente de interés y la selección de los agentes reductores y estabilizantes, como el agente reductor inicia el proceso agregación, se busca que el agente estabilizante proteja rápidamente la partícula formada para mantenerla dentro del rango de los nanómetros (Szczyglewska et al., 2023). Dentro de los agentes reductores más comunes se encuentran ácido cítrico, glucosa, etilenglicol, acido oxálico, acido ascórbico y peróxido de hidrogeno. Mientras que dentro de los agentes reductores más comunes se encuentra el uso de ácido oleico, acido linoleico, polivinilpirrolidona (PVA), alcohol polivinílico, polietilenglicol, así como el uso de surfactantes como Tween® 20 y Tween® 80 (Szczyglewska et al., 2023). En la Figura 3 se ejemplifica el proceso de obtención de AgNPs, aunque este aplique también para otros metales mediane reducción química o biológica, usualmente a las nanopartículas obtenidas a partir de reducción biológica se les denomina como fitonanopartículas o nanopartículas verdes. En el caso de la ruta de fitosíntesis de aprovechan los grupos funcionales de los fitocompuestos de extractos de origen vegetal o de cultivos de microorganismos para la reducción y estabilización de las nanopartículas verdes (Chaudhary et al., 2023).

Figura 3. Obtención de Nanopartículas de Plata (AgNPs) a partir de reducción química y reducción biológica (Guilger-Casagrande and Lima, 2019; Hamida et al., 2020; Jabeen et al., 2021; Kumari et al., 2023; Szczyglewska et al., 2023).



Las nanopartículas, también puede clasificarse dependiendo de la composición de la partícula, por ejemplo, se pueden mencionar nanopartículas orgánicas, inorgánicas y nanocompositos. Dentro de las nanopartículas orgánicas se pueden encontrar las partículas poliméricas a base de quitosano, maltodextrina, alginato, liposomas, albumina, celulosa, pectina, polivinilpirrolidona, entre otras. Mientras que en las inorgánicas las partículas de silicio (SiNPs), cobre (CuNPs), zinc (ZnNPs), plata (AgNPs), selenio (SeNPs), oro (AuNPs), entre otros. Mientras que, en los nanocompositos, se habla de la interacción de al menos 2 tipos de materiales como inorgánicos, orgánicos y metálicos. Aunque dentro de las nanopartículas orgánicas podrían clasificarse las estructuras de carbono, estas suelen tener su propia clasificación, las nanopartículas a base de carbono, dentro de las cuales se pueden encontrar los fulerenos, fibras de carbono, nanotubos de carbono y grafeno (Joudeh and Linke, 2022; Mekuye and Abera, 2023).

Dentro de las nanopartículas inorgánicas, una de las de mayor interés de investigación son las nanopartículas de silicio, que puede obtenerse a partir de ambas rutas de síntesis y de diversos precursores como especies de silicatos completamente inorgánicos como el metasilicato de sodio o con partes orgánicas como el tetraetilortosilicato, el principal precursor en las metodologías de Sol-Gel. En cuanto a sus campos de aplicación, pueden encontrarse desarrollos en áreas de medicina, aeroespacial, textiles, farmacología y agronomía, principalmente debido a la gran versatilidad de las nanopartículas, tanto en cuanto a sus propiedades como a sus posibles morfologías y modificaciones (Chen et al., 2009; Craddock, 2018; Kappel et al., 2014; Kuroda et al., 2014; Moretto et al., 2000; Pape, 2011).

Por ejemplo, las nanopartículas de sílice pueden obtenerse desde esferas sólidas, hasta como esferas con núcleo diferente a la sílice, así como pueden obtenerse esferas mesoporosas que permite la encapsulación y liberación controlada con activos de interés, a su vez, las tres estructuras mencionadas pueden modificarse o funcionalizarse según su aplicación, tal puede ser la deposición de cationes metálicos para el control de diferentes microorganismos o la adición de surfactantes para mejorar la estabilidad de una suspensión o emulsión, e incluso la deposición de un fármaco de interés para asegurar su liberación paulatina (Chen et al., 2009; Craddock, 2018; Kappel et al., 2014; Kuroda et al., 2014; Moretto et al., 2000; Pape, 2011; Pawlenko, 2011).

TÉCNICAS DE CARACTERIZACIÓN

Dynamic Light Scattering

La medición y el control del tamaño de las partículas a escala nanométrica son fundamentales, ya que el tamaño puede influir significativamente en las propiedades físicas y químicas de los nanomateriales (Joudeh y Linke, 2022). Una de las herramientas más utilizadas es la técnica de dispersión dinámica de luz (DLS) por sus siglas en inglés, es una técnica fisicoquímica no invasiva empleada de manera amplia en las investigaciones de nanomateriales, con el objetivo de determinar la distribución del tamaño hidrodinámico que va de nanómetros hasta micrómetros, de manera rápida, económica y con alta precisión estadísticamente (Jia et al., 2023). El método DLS utiliza el principio del movimiento browniano aplicado para muestras en solución, solución coloidal o suspensión. Este movimiento aleatorio debido a las colisiones es más rápido para partículas pequeñas y lento para partículas grandes (Segrè, Behred y Pusey, 1995).

El principio de la técnica DLS se representa en la Figura 4. En ella se observa que, al incidir un haz de luz láser sobre una suspensión coloidal, las partículas dispersan la luz de forma variable a lo largo del tiempo debido a su movimiento browniano. La intensidad de la luz dispersada es registrada por un detector, a un ángulo de dispersión determinado, y estas variaciones están relacionadas con el tamaño hidrodinámico de las partículas. A partir del análisis de las variaciones es posible estimar el tamaño promedio y la distribución de tamaños mediante la ecuación de Stokes-Einstein (Segrè, Behred y Pusey, 1995).

La técnica DLS posee un amplio potencial analítico, ya que permite determinar el índice de polidispersidad (PDI), un parámetro clave para evaluar la uniformidad y estabilidad de la distribución de tamaños de las partículas en suspensión. Por ejemplo, un PDI inferior a 0.05 indica una muestra monodispersa con alta uniformidad, mientras un PDI superior a 0.7 indica que la muestra es polidispersa, con una distribución de tamaño de partícula muy amplia, lo que sugiere una alta heterogeneidad, que podrían reflejar agregación en la muestra (Valdez-Salas et al., 2020).

Es importante resaltar que el DLS acoplado a un sistema de ELS (Electroforesis por dispersión de luz), mide el potencial Z. Este parámetro está relacionado con la estabilidad coloidal, ya que proporciona una estimación del comportamiento electrocinético de las nanopartículas en suspensión. Valores de potencial Z por encima de ± 30 mV suelen considerarse indicativos de una buena estabilidad, debido a las fuerzas de repulsión electrostáticas que evitan la agregación y sedimentación de las partículas (Akram et al., 2024; Bhattacharjee, 2016).

Microscopia electrónica de barrido

La caracterización por microscopia electrónica de barrido de alta resolución (SEM) tiene como objetivo estimar el tamaño y analizar la morfología de los nanomateriales. Esta técnica tiene amplias aplicaciones en ciencias de materiales, agricultura, biotecnología y procesos de nanofabricación. A través de SEM se producen imágenes de alta resolución a partir de electrones secundarios y/o retrodispersados generados mediante la proyección del haz de electrones primarios enfocados sobre la superficie de una muestra (Lee et al., 2021), cómo se observa en la representación de SEM en la Figura 4.

Los microscopios electrónicos de barrido pueden estar equipados con un espectrómetro de dispersión de energía de rayos X (EDS), que permite analizar la composición elemental de una muestra. Cuando la superficie de la muestra es bombardeada por el haz de electrones primarios del SEM a energías de hasta 30 keV, se generan rayos X característicos como resultado de la ionización de electrones en capas internas de los átomos, estas emisiones son detectadas y analizadas por el sistema EDS. Esta información permite identificar y cuantificar los elementos presentes en la muestra (Vladár y Hodoroaba, 2020; Hordoroaba, 2020).

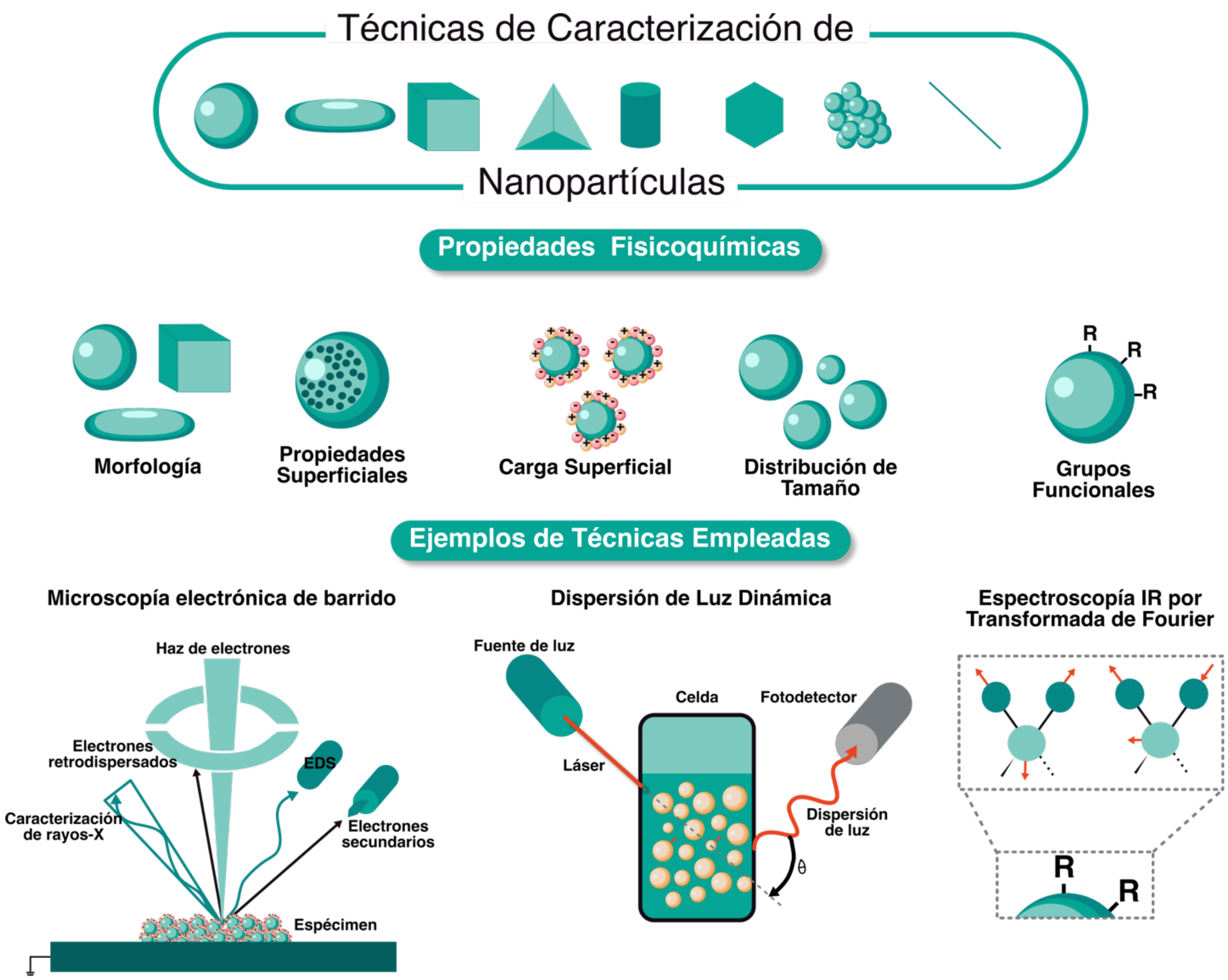
Espectroscopía Infrarroja por Transformada de Fourier

La espectroscopía infrarroja se basa en la absorción de radiación infrarroja por parte de las moléculas, lo que provoca la excitación a los modos vibracionales de sus enlaces químicos entre átomos. Cada tipo de enlace absorbe energía en una región específica del espectro, lo que permite identificar los grupos funcionales presentes en una muestra en estado sólido, líquido o gaseoso mediante su firma espectral característica (Pasieczna-Patkowska et al., 2023). La espectroscopía infrarroja por transformada de Fourier (FT-IR) mide en una región del espectro de 4000 a 400 cm^{-1}. Por ejemplo, los grupos hidroxilo (–OH) en la región del espectro 4000–2500 cm^{-1}, banda ancha única, los carbonilos (C=O) región 2000–1500 cm^{-1} y los carboxilos

(–COOH) 1710-1750 cm^{-1} y una banda ancha 2500–3500 cm^{-1} (Fiore y Pellerito, 2021).

Por lo tanto, la técnica de FT-IR permite identificar y confirmar la presencia de grupos funcionales o moléculas específicas en la superficie de las nanopartículas, lo que resulta importante para evaluar su funcionalización y sus posibles aplicaciones. Además, es útil para identificar los grupos funcionales principales en extractos vegetales con potencial reductor, comúnmente utilizados en síntesis verde de nanopartículas.

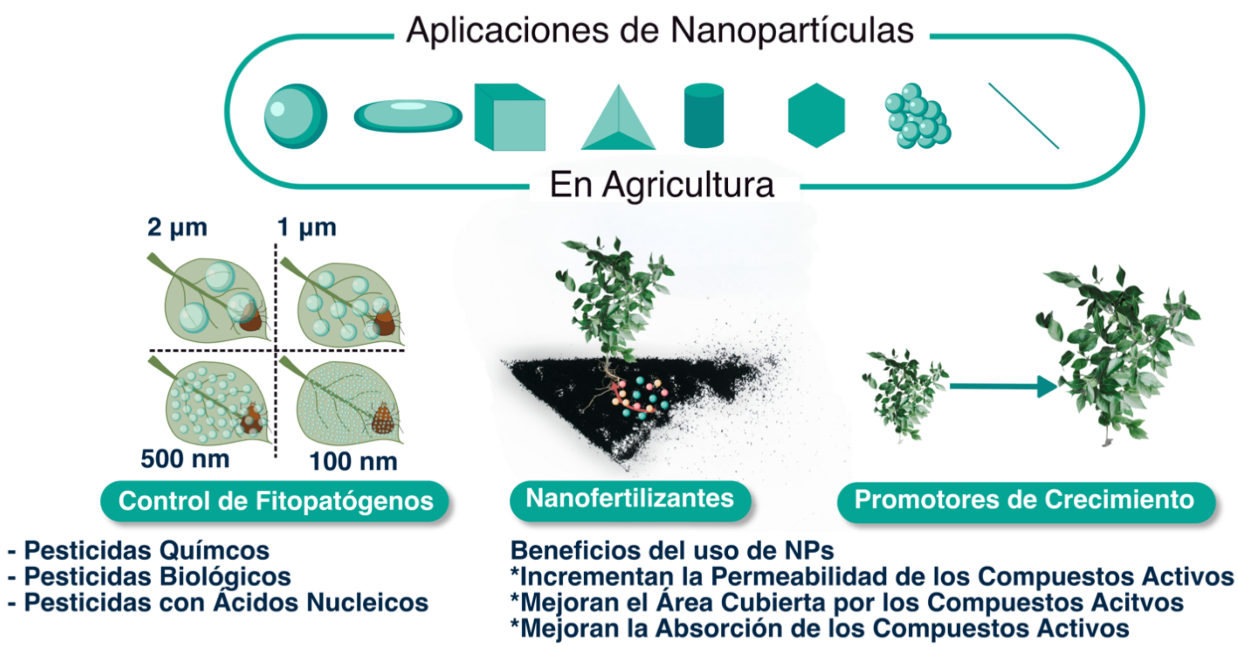
Figura 4. Técnicas principales de caracterización de nanopartículas (Giurlani et al., 2020; Modena et al., 2019; Mourdikoudis et al., 2018; Pasieczna-Patkowska et al., 2025; Todaro and Santi, 2022)



APLICACIONES EN AGRICULTURA

La agricultura moderna enfrenta múltiples desafíos que comprometen la sostenibilidad de la producción de alimentos, entre ellos el cambio climático, la creciente resistencia a plagas y enfermedades, y el agotamiento de los recursos naturales. En este sentido, la nanotecnología ha emergido como una alternativa prometedora, al permitir la creación y aplicación de nanopartículas con un amplio potencial para revolucionar la agricultura contemporánea (Figura 5). Estos nanomateriales, con dimensiones inferiores a 100 nm, presentan propiedades fisicoquímicas únicas, como una mayor relación superficie-volumen, mayor reactividad y la capacidad de transportar sustancias activas de manera dirigida (Khan et al., 2019).

Figura 5. Potencial de las nanopartículas en la agricultura: aplicaciones para control de patógenos, nanofertilizantes y liberación controlada en cultivos. Adaptado de An et al. (2022)



En el ámbito agrícola, las NPs han ganado popularidad por su potencial para actuar como fertilizantes de liberación controlada, estimulantes del crecimiento vegetal, agentes biofortificantes y soluciones eficaces para el manejo de enfermedades, plagas y condiciones de estrés abiótico (Fincheira et al., 2023).

Esta sección presenta una revisión de sus principales aplicaciones en la agricultura, organizadas en cinco áreas clave: estimulación del crecimiento vegetal, control de enfermedades, manejo de plagas, biofortificación y modulación de rutas fisiológicas y moleculares.

A. Nanopartículas como bioestimulantes del crecimiento vegetal

Las NPs pueden actuar como bioestimulantes al potenciar procesos fisiológicos como la fotosíntesis, la absorción de nutrientes y la resistencia al estrés abiótico. El tamaño nanométrico de estos compuestos permite una mejor penetración celular y una liberación más eficiente de los elementos activos (Pramanik et al., 2020).

Diversos estudios han demostrado que nanopartículas como las de óxido de zinc (ZnO), óxido de hierro (Fe_2O_3) y dióxido de titanio (TiO_2) pueden inducir respuestas positivas en el crecimiento de las plantas al estimular la producción de clorofila, enzimas antioxidantes y regulación hormonal (Rastogi et al., 2019). Por ejemplo, el uso de ZnO NPs ha mostrado incrementar la longitud de raíces y la biomasa aérea en cultivos como trigo y arroz, lo cual sugiere su potencial para mejorar la productividad agrícola en condiciones limitantes (Qu et al., 2022; Hashmi et al., 2024). Estas NPs pueden actuar directamente sobre rutas metabólicas clave, regulando hormonas vegetales como auxinas, citoquininas y giberelinas, o bien, mejorando la absorción de nutrientes esenciales, además, la aplicación controlada de las nanopartículas permite una respuesta sostenible y eficiente frente a condiciones adversas, posicionándolas como una herramienta prometedora para aumentar la productividad agrícola sin comprometer al ecosistema (Upadhayay et al., 2023).

B. Aplicación de NPs en el control de enfermedades fúngicas, bacterianas y virales

Las nanopartículas metálicas poseen propiedades antimicrobianas que pueden ser utilizadas para el control de fitopatógenos sin recurrir a fungicidas o tratamientos convencionales. Nanopartículas de plata, cobre, óxido de zinc (ZnO NPs) y óxido de magnesio (MgO NPs) han demostrado una actividad significativa contra hongos como *Fusarium* spp., *Botrytis cinerea* y *Alternaria solani,* así como bacterias fitopatógenas como *Pseudomonas syringae* y *Xanthomonas campestris* (Kashyap et al., 2021).

La síntesis verde de AgNPs, utilizando extractos vegetales, ha demostrado ser una estrategia efectiva para el control de patógenos agrícolas en frutos durante la etapa de poscosecha. Las AgNPs también se utilizan en envases para prevenir el daño de los productos alimenticios causado por patógenos (Siddiqi et al., 2018). Del mismo modo, presentan una actividad antimicrobiana significativa, al igual que la como también las CuNPs y otros óxidos metálicos. Otra ventaja es la posibilidad de combinar nanopartículas con extractos vegetales o feromonas para formular bioplaguicidas de liberación lenta, con alta especificidad y menor impacto ambiental (Hernández-Hernández et al., 2020). Esta aproximación representa una alternativa prometedora ante la creciente resistencia a los insecticidas sintéticos.

En cultivos de tomate y trigo, las NPs de óxido de zinc y cobre han reducido significativamente el desarrollo de enfermedades causadas por *Fusarium, Alternaria, Botrytis cinerea* y *Xanthomonas* (González-Merino et al., 2021; Tryfon et al., 2021; Mosa et al., 2023). La acción de las NPs como agentes protectores ofrece una alternativa sostenible frente al uso excesivo de pesticidas convencionales

(Machado et al., 2023). Estos nanomateriales pueden inactivar patógenos fúngicos y bacterianos mediante la generación de especies reactivas de oxígeno (ROS), el daño a la pared celular o la interferencia con el ADN microbiano (Hamida et al., 2020). Asimismo, su bajo impacto ambiental y eficacia a bajas concentraciones las convierten en una opción atractiva para la agricultura sustentable (Atanda et al., 2025).

C. Nanopartículas en el control de insectos plaga

La toxicidad inherente de ciertas nanopartículas puede ser aprovechada para el manejo de insectos, mediante mecanismos tanto físicos como bioquímicos. Por ejemplo, las AgNPs se emplean ampliamente en la formulación de biopesticidas, debido a capacidad de liberación controlada y sostenida, lo cual permite una eficiencia prolongada. Las AgNPs son consideradas una de las opciones más rentables y eficientes para la reducción catalítica de contaminantes orgánicos, y su efectividad frente a insectos se atribuye a diversas propiedades fisicoquímicas, como el tamaño de partícula, estructura superficial, cristalinidad, carga superficial y actividad catalítica, así como a la concentración utilizada. Estas características mejoran su capacidad para atravesar barreras biológicas e inducir efectos fisiológicos adversos, incluyendo daños físicos que comprometen la integridad estructural y funcional de los tejidos de los insectos (Martínez-Cisterna et al., 2024; Alian et al., 2025).

Otra ventaja, es la posibilidad de combinar nanopartículas con extractos vegetales o feromonas para la formulación de bioplaguicidas de liberación lenta, con alta especificidad y bajo impacto ambiental (Manna et al., 2023), esta aproximación representa una alternativa prometedora ante la resistencia creciente a los insecticidas sintéticos.

La plata y el óxido de zinc, aplicados en formulaciones coloidales, han demostrado provocar mortalidad en insectos plaga como *Spodoptera litura* (Elmasry, 2021), *Helicoverpa armigera* (Asghar et al., 2022) y *Bemisia tabaci* (Taheri et al., 2020). Los mecanismos de acción incluyen la generación de ROS, interferencia con el sistema digestivo y daño en el sistema nervioso central de los insectos (Martínez-Cisterna et al., 2024). Además, el uso de NPs posibilita una liberación controlada del ingrediente activo y mejora su estabilidad frente a la degradación ambiental (Li et al., 2025).

D. Biofortificación con nanopartículas: mejora del contenido nutricional de los alimentos

La biofortificación consiste en incrementar el contenido de nutrientes esenciales en los cultivos, con el objetivo de mejorar su valor nutricional. Las nanopartículas ofrecen una vía eficiente para la entrega controlada y dirigida de micronutrientes como zinc, hierro, selenio y cobre, elementos esenciales para la salud humana y vegetal (Zahid et al., 2025). Las nanopartículas de hierro (Fe NPs) han sido utilizadas exitosamente en cultivos como arroz, espinaca y lentejas, incrementando su contenido de hierro biodisponible sin generar efectos tóxicos en las plantas o en el ambiente. Asimismo, las nanopartículas de óxido de zinc (ZnO NPs), aplicadas al suelo o por vía foliar en cultivos de arroz, trigo y maíz, han demostrado mejorar significativamente el contenido de zinc en grano sin afectar negativamente el rendimiento (Rastogi et al., 2019).

El reducido tamaño de las nanopartículas favorece su absorción y transporte dentro de la planta, lo que incrementa su eficiencia en comparación con los fertilizantes convencionales (Siddiqi et al., 2018). Además, su aplicación precisa reduce las pérdidas por lixiviación y minimiza el impacto ambiental, lo cual contribuye a una agricultura más sustentable.

E. Modulación de rutas fisiológicas y moleculares mediante nanopartículas

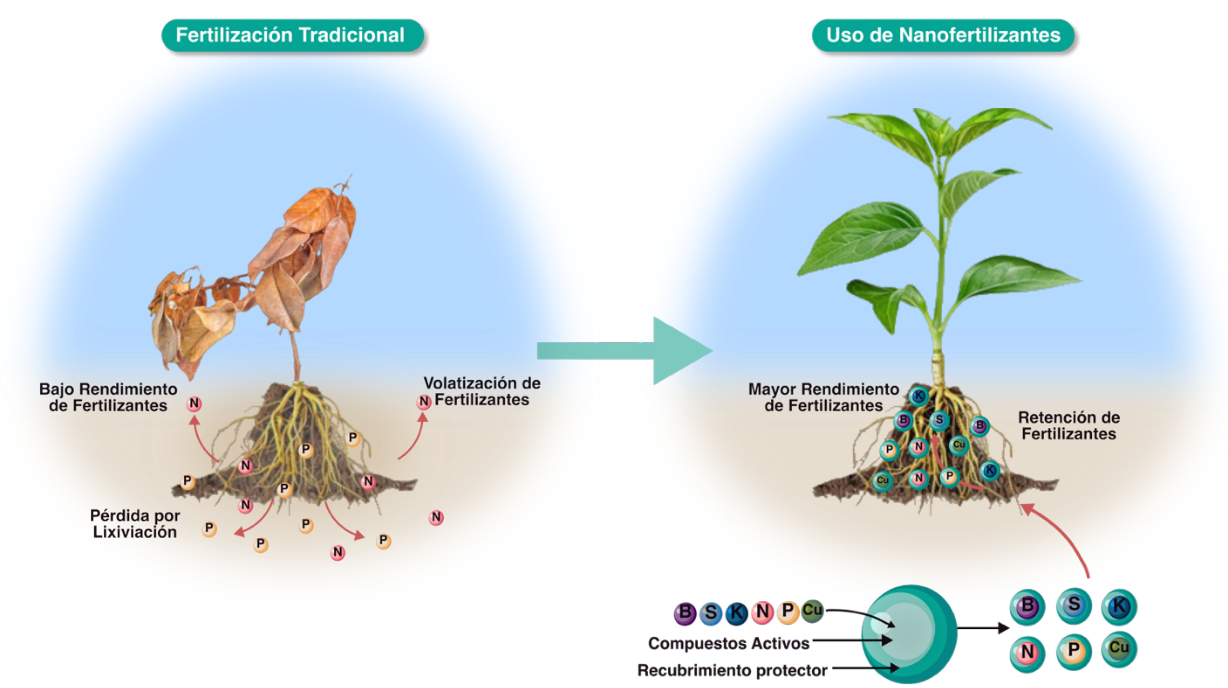
Las nanopartículas pueden inducir la activación de rutas metabólicas y fisiológicas involucradas en la producción de metabolitos secundarios y en el fortalecimiento de los mecanismos de defensa vegetal. En cultivos sometidos a estrés abiótico, las ZnNPs, CuNPs y SiNPs han demostrado estimular la expresión de genes asociados al sistema antioxidante, promoviendo la síntesis de compuestos como lignina, fenoles y flavonoides, los cuales cumplen un papel crucial en la respuesta adaptativa (Morales-Quintana, 2023). Asimismo, las NPs pueden modular los niveles de fitohormonas relacionadas con la resistencia al estrés, como el ácido abscísico, el etileno y el ácido salicílico, mejorando así la capacidad de adaptación de las plantas (Tripathi et al., 2022). Esta activación de rutas moleculares específicas contribuye a incrementar la tolerancia de los cultivos frente a condiciones adversas, incluyendo salinidad, sequía y temperaturas extremas.

F. Liberación controlada de agroquímicos mediante NPs

Una de las aplicaciones más prometedoras de las nanopartículas en la agricultura es su uso como vehículos inteligentes para la liberación controlada de agroquímicos. Mediante la encapsulación de ingredientes activos (como fertilizantes, herbicidas o pesticidas) dentro de estructuras a escala nanométrica, es posible lograr una liberación sostenida, dirigida y localizada del compuesto, lo cual incrementa su eficacia y disminuye significativamente el impacto ambiental.

Para el diseño de estos sistemas de liberación se emplean polímeros biodegradables como el quitosano, así como matrices metálicas o híbridas, que permiten una formulación estable y eficiente (Figura 6). Además, estas nanopreparaciones pueden ser sensibles a estímulos ambientales específicos como el pH, la temperatura o la humedad, lo que posibilita una liberación regulada del agroquímico en función de las necesidades fisiológicas del cultivo y las condiciones del entorno.

Figura 6. Uso de nanopartículas como sistemas de liberación controlada de nutrientes en la agricultura. Adaptado de An et al. (2022)



Asimismo, estas formulaciones poseen la capacidad de responder a señales ambientales del entorno agrícola, permitiendo que el agroquímico sea liberado en el momento y lugar óptimos para su absorción por la planta. Estas aplicaciones no solo inciden en la mejora fisiológica y bioquímica de las plantas, sino que también permiten reducir la dependencia de insumos agroquímicos convencionales. A pesar de los avances, aún existen retos importantes por abordar, ya que las NPs representan una herramienta innovadora para enfrentar los desafíos actuales de la agricultura, como el cambio climático, la degradación de los suelos y la necesidad de incrementar la producción de alimentos nutritivos y sostenibles.

G. Nanopartículas y fitotoxicidad.

En agronomía, el uso de nanopartículas puede inducir efectos tanto beneficiosos como adversos en el crecimiento y desarrollo de las plantas. La naturaleza química y fisicoquímica de las NPs, la tolerancia del organismo vegetal y la vía de exposición son factores determinantes en la respuesta fisiológica y bioquímica generada (Rico et al., 2015; Farooq et al., 2023; Hassanisaadi et al., 2022).

Las propiedades que pueden definir la fitotoxicidad de las NPs son la dosis empleada, tamaño, morfología, porosidad, carga superficial, naturaleza química, vehículo y vía de exposición que puede ser vía foliar o radicular principalmente. Por lo que, la respuesta del organismo dependerá de la vía o mecanismos de entrada de las NPs al organismo, la cual se propone en la literatura que puede ser mediante tres vías principales y su absorción o traslocación puede ser mediante mecanismos activos o pasivos, cuando las nanopartículas logran entrar a la planta, estas pueden movilizarse a través de la vía simplástica o apoplástica hasta llegar al xilema pero su entrada no asegura su libre movilidad, estas pueden aglomerarse o acumularse en diferentes órganos de la planta (Rico et al., 2015 Farooq et al., 2023; Hassanisaadi et al., 2022)

Dentro de los indicadores de respuesta fisiológica se pueden encontrar los cambios de germinación de semillas, así como en producción de biomasa, rendimiento de cultivo y en el sistema radicular, mientras que dentro de los indicadores bioquímicos se pueden encontrar daños en el material proteico y genético (así como la expresión o supresión de algunos genes), estrés oxidativo y cambios en actividad fotosintética (Rico et al., 2015; Farooq et al., 2023; Hassanisaadi et al., 2022). En los indicadores bioquímicos se encuentran alteraciones en actividades enzimáticas, contenido de clorofila I y II, así como alteraciones en el contenido metabolitos primarios como proteínas y carbohidratos, en metabolitos secundarios como ácido cafeico y ácido clorogénico, los que se sintetizan a partir de la actividad de la enzima fenilalanina amonio liasa (PAL) (Farooq et al., 2023; Joya Dávila et al., 2023; Luján-Hidalgo et al., 2020). Dentro de estas alteraciones, se puede generar estrés oxidativo como respuesta ante la presencia de ciertas nanopartículas, así como la interferencia con el rendimiento de los fotosistemas al absorber energía proveniente de clorofila I y II (Tryfon et al., 2024). Las plantas suelen producir especies reactivas de oxígeno (ROS) de manera regular ya sea como subproductos de su metabolismo aeróbico o como respuesta ante factores de peligro (estrés) bióticos o abióticos, que pueden intervenir de forma tanto benéfica como dañina en las plantas, las cuales mantienen un balance “saludable” mediane mecanismos antioxidantes que pueden seguir rutas químicas o enzimáticas.

Dentro de las rutas químicas se pueden encontrar ascorbato, glutatión, fenoles, tioles, tocoferol, entre otros, mientras que dentro de las enzimas que intervienen dentro de esta regulación se pueden encontrar el superóxido dismutasa (SOD), CAT, peroxidasas como ascorbato peroxidasa (APX) y guayacol peroxidasa (Farooq et al., 2023; Joya-Dávila, 2023; Tryfon et al., 2024). A niveles moderados de ROS en las plantas se puede mantener un balance entre las ROS producidas, utilizadas y neutralizadas, las ROS pueden interferir de manera positiva como segundos mensajeros en reacciones de cadena intercelular que regulan la respuesta de células ante diferentes estímulos, como la activación de sus mecanismos de defensa ante la presencia de patógenos, cambios en la conductividad estomacal, respuestas de hipersensibilidad y desarrollo de tolerancia ante factores de estrés bióticos y abióticos (Farooq et al., 2023). Cuando este balance excede los límites funcionales de las plantas, estos pueden generar daño oxidativo a biomoléculas mediante perdida de electrolitos, peroxidación lipídica, produciendo malondialdehido como subproducto de la oxidación de los fosfolípidos de la membrana celular, además de causar oxidación de proteínas mediante la desintegración de enlaces aminos de la cadena polipeptídica, la modificación de la carga superficial de las proteínas que puede causar aglomeraciones y acelerar su degradación o inactivación, así como la degradación del material genético mediante la oxidación de la desoxirribosa causando desnaturalización del DNA (Farooq et al., 2023).

Dentro de indicadores o alteraciones bioquímicas que pueden implicar la aplicación de nanopartículas en plantas se encuentran la modificación del comportamiento de los fotosistemas por lo que la técnica de fluorescencia de clorofila puede funcionar como indicador de estrés inducido por nanopartículas.

La fotosíntesis inicia por la incidencia y absorción de energía dentro de la radiación fotosintéticamente activa (PAR), de la cual un porcentaje es absorbida por los pigmentos fotosintéticos como clorofila *a* y *b* junto con carotenoides provocando su excitación, misma, que puede liberarse a partir de tres rutas, por lo que los pigmentos fotosintéticos logran volver a su estado basal o de reposo, parte de la energía absorbida logra aprovecharse químicamente mediante la donación o transferencia a otras moléculas cercanas con la capacidad de aceptar los electrones, otra parte de esta energía se libera en forma de calor y la menor parte de esta en forma de energía lumínica o fluorescencia, como los tres mecanismos de transferencia de energía son complementarios, a partir de la medición de uno de estos mecanismos, se puede obtener información sobre los demás (Moreno et al., 2008).

El 97% de la energía absorbida suele ser utilizada durante el evento fotoquímico primario que se consta de la oxidación de una molécula de clorofila *a* (Chl a^+) al perder o donar un electrón para volver a su estado basal, mismo que es aceptado durante la reducción de feofitina ($Pheo^-$) mientras que el 2.5% se libera en forma de calor y el 0.5% restante en fluorescencia roja. Cuando el evento fotoquímico primario no ocurre, se libera en forma de calor entre el 95 al 97% de la energía incidente y del 2.5 al 5% como fluorescencia roja, lo que permite hacer mediciones sobre el comportamiento de los fotosistemas en plantas (Moreno et al., 2008).

Particularmente, cuando se habla de fluorescencia de clorofila, se habla de la actividad de la clorofila *a* de los fotosistemas II ya que en comparación con los fotosistemas II, el fotosistema I emite significativamente menor energía a 25 °C (Moreno et al., 2008). Como el comportamiento y rendimiento de los fotosistemas II se puede ver a afectado de manera directa e indirecta por factores bióticos y abióticos, la fluorescencia de clorofila ayuda a determinar posibles mecanismos de respuesta ante estos factores (Moreno et al., 2008). Uno de los resultados que se pueden obtener a partir de fluorescencia de clorofila es la cinética de Kautsky que se puede dividir por una fase rápida (O, J, I, P) donde la fluorescencia incrementa rápidamente después de la iluminación de los fotosistemas durante los eventos primarios del PSII, produciendo una reducción la quinona A (Q_A) y la plastoquinona (PQ), donde principalmente la Q_A limita la actividad de los centros de reacción, ya que cuando la Q_A se encuentra en su estado reducido se dice que el centro se encuentra "cerrado" mismo que no puede "abrirse" hasta alcanzar su oxidación al donar los electrones que lo mantienen en estado de excitación a la quinona B (Q_B). Mientras que la Q_A se mantenga reducida ocurre un decremento en la eficiencia fotoquímica y aumenta la energía liberada mediante fluorescencia (Moreno et al., 2008).Seguido continua una fase lenta de decremento de fluorescencia (P, S, M T) que se produce principalmente por dos mecanismos, a uno de estos se le conoce como decaimiento fotoquímico (qP) que se debe a la oxidación de la QA, al segundo se le conoce como decaimiento no fotoquímico (qNP) que se debe principalmente por el cambio del gradiente de potencial electroquímico protónico transtilacoidal. Los cambios en el valor de qNP ayudan a entender cambios en gradientes de pH, fotoinhibición, reducción del rendimiento de los PSII (ϕ_{PSII}), así como la velocidad del transporte de electrones fotosintéticos (ETR) (Moreno et al., 2008).

La fluorescencia de clorofila es una técnica de medición no invasiva de la actividad del fotosistema II (PSII) que se suele utilizar en estudios de fisiología vegetal ya que se puede relacionar con el impacto de factores bióticos y abióticos sobre los PSII, por lo que la aplicación de fluorescencia de clorofila es de utilidad para analizar como las plantas responden ante diferentes estímulos como cambios ambientales, variación genética y diversidad del ecosistema (Murchie and Lawson, 2013).

RESULTADOS DE INVESTIGACIÓN APLICADA CON NANOPARTÍCULAS

Diversos estudios han demostrado que ciertas nanopartículas (NPs) promueven el crecimiento vegetal. En la tabla 1 se resumen investigaciones que respaldan el potencial de las NPs como bioestimulantes, biofortificantes y agentes de biocontrol. Sin embargo, es importante considerar que su eficacia depende de la dosis, tipo de nanomaterial y especie vegetal. El uso de NPs también puede contribuir a mitigar deficiencias nutricionales, especialmente en suelos empobrecidos (Khan et al., 2019), así como mejorar la biodisponibilidad de insumos agrícolas reduciendo pérdidas por lixiviación y volatilización (Ficheira et al., 2023; An et al., 2022). También es importante evaluar de manera integral los efectos negativos en el desarrollo de los cultivos, así como las implicaciones ecológicas, por último y no menos importante actualizar y establecer marcos regulatorios para la adopción segura de nanopartículas con aplicación agrícola.

Tabla 1. Avances recientes en aplicación de nanopartículas en sistemas agrícolas.

Nanopartícula	Base de estudio	Tipo de síntesis	Concentración	Efectos positivos	Efectos negativos	Fuente
Nanopartículas como bioestimulantes y biofortificación en el crecimiento vegetal						
Hidroxiapatita (nHAp)	Semilla *Cucumis sativa*	Precipitación húmeda	25, 50, 100, 200, 500, 1 000 y 2 000 mg L^{-1}	Germinación, vigor y longitud de radícula (25 mg L^{-1})	Mayores efectos inhibidores a >200 mg L^{-1}	López-Martínez et al., 2023
Óxido de hierro (IONPs)	*Vigna radiata*	Verde (extracto de *Syzigium. cumini*)	100, 250 y 500 mg kg^{-1} suelo. Uso de inoculante *Rhizobium pusense*	Longitud de raíz y tallo, biomasa seca, clorofila y contenido proteico	Sin fitotoxicidad	Saleem y Khan., 2023
CuONPs	Frutos de melón	Verde (cáscara de naranja)	150, 200, 250, 300 y 350 mg L^{-1}	Rendimiento, firmeza y compuestos bioactivos a 150 mg L^{-1}	En dosis altas rendimiento y compuestos fitoquímicos	Buendía-García et al., 2025
Aplicación de NPs en el control de enfermedades fúngicas, bacterianas y virales						
AgNPs	*Cajanus cajan* (proteína aislada) + goma de *Tamarindus indica*	Verde (extracto de hojas de *Annona muricata*)	25 % AgNPs en los recubrimientos formulados	Estructura mecánica y permeabilidad al vapor de agua, aplicación en postcosecha.	Oscurecimiento en las películas.	Estudillo-Díaz et al. 2023
Nanopartículas de plata sintetizadas por *Desertifilum sp.* (D-SNP)	*E. coli* *S. Typhimurium* *S. Mutans* *K. Pneumoniae* *Staphylococcus aureus*	Verde (extracto de *Desertifilum sp.)*	0.3, 0.6, 0.9, 1.2, 1.5, y 1.8 mg mL^{-1}	-Potencial frente a bacterias multirresistentes. -Alteraciones en enzimas antioxidantes y cambios morfológicos	–	Hamida et al., 2020
Nanopartículas en el control de insectos plaga						

ZnONPs	*Spodoptera litura* *Aphis gossypii*	Verde (clara de huevo)	Thioxam 25%WG/ZnONPs, Actara 25%WG/ZnONPs	Eficacia insecticida: 28–39% mortalidad adicional	–	Elmasry, 2021
AgNPs, ZnONPs	*Helicoverpa armigera*	Verde (extracto de Neem)	100 – 200 ppm	Mortalidad: 96 % (AgNPs), 82 % (ZnONPs)	–	**Asghar** et al., (2022)
Modulación de rutas fisiológicas y moleculares mediante nanopartículas						
ZnONPs, FeNPs y SiO_2NPs	Plantas bajo estrés hídrico	Verde y química	No indicadas	Inducen genes antioxidantes, tolerancia a la sequía y regula fitohormonas	Riesgos por acumulación	Rasheed et al., (2022)
ZnONPs	*Cucumis melo* L. (melón) bajo estrés por sequía	Verde (extracto de *A. annua*)	75, 100, 125 y 150 mg L^{-1}	Tolerancia a sequía (Expresión de genes), actividad antioxidante y absorción de nutrientes (75 y 100 mg L^{-1})	La tolerancia fue limitada a concentraciones altas	Rehman et al., 2023

CONCLUSIONES

El uso de nanopartículas en agronomía ofrece una amplia gama de aplicaciones derivadas de la diversidad de rutas de síntesis (físicas, químicas y biológicas), materiales base y funcionalizaciones posibles que la nanotecnología proporciona. Estas rutas impactan directamente en sus propiedades fisicoquímicas, como tamaño, forma, carga y funcionalidad superficial, aspectos que deben caracterizarse mediante técnicas como DLS, SEM y FTIR para asegurar su estabilidad y efectividad en campo.

En el ámbito agrícola, las nanopartículas han demostrado potencial como bioestimulantes, agentes de control contra patógenos (fúngicos, bacterianos y virales), moduladores de rutas fisiológicas y moleculares, herramientas para la biofortificación de cultivos y vehículos para la liberación controlada de agroquímicos. No obstante, su implementación debe ser cuidadosamente diseñada y adaptada al tipo de cultivo y condiciones específicas, ya que la interacción nanomaterial-planta-patógeno es compleja y puede generar efectos no deseados en la microbiota benéfica del entorno.

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CAPÍTULO 3. La roya del café (*Hemileia vastatrix*) y estrategias actuales de control

Título original del artículo: Coffee Leaf Rust (*Hemileia vastatrix*) Disease in Coffee Plants and Perspectives by the Disease Control

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Este capítulo corresponde a un artículo publicado en: *Phyton, International Journal of Experimental Botany*, Vol. 94(1), 2025, pp. 57–67. DOI: https://doi.org/10.32604/phyton.2025.049612 *(El contenido se presenta en su idioma original con fines académicos).*

Resumen

La roya del café (*Hemileia vastatrix*) es uno de los fitopatógenos más destructivos para las plantaciones de *Coffea arabica*, afectando tanto la productividad como la calidad de taza. Este capítulo presenta una revisión sobre la situación actual de la roya en México, incluyendo su ciclo de patogenicidad y las interacciones planta-patógeno que determinan la compatibilidad o resistencia. Se analizan las rutas de defensa activadas por la planta hospedera y las principales estrategias actuales de control, incluyendo acciones fitosanitarias, control biológico y químico. Finalmente, se discuten nuevas perspectivas de manejo mediante el uso de nanopartículas y tecnologías emergentes.

Palabras clave: Roya del café; *Coffea arabica*; patogenicidad; nanopartículas; control biológico.

Abstract

Coffee Leaf Rust (CLR) is caused by *Hemileia vastatrix* in *Coffea* spp. It is one of the most dangerous phytopathogens for coffee plantations in terms of coffee productivity and coffee cup quality. In this review, we resume the problem of CLR in Mexico and the pathogenesis of *H. vastatrix*. The review abord plant-pathogen interactions which lead a compatible or incompatible interactions and result in CLR disease or resistance, respectively. The review abord Coffea spp. defense response pathways involved in *H. vastatrix* pathogenicity. Additionally, current measures to control *H. vastatrix* proliferation and germination were aborded focused on phytosanitary actions, and biological and chemical control. Finally, new trendlines to reduce the impact of CLR as nanoparticles and nanotechnology were analyzed.

Keywords: Coffee leaf rust; Coffea arabica; pathogenesis; nanoparticles; biological control.

Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2024.049612

REVIEW

Coffee Leaf Rust (*Hemileia vastatrix*) Disease in Coffee Plants and Perspectives by the Disease Control

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Received: 12 January 2024 Accepted: 02 April 2024 Published: 28 May 2024

ABSTRACT

Coffee Leaf Rust (CLR) is caused by *Hemileia vastatrix* in *Coffea* spp. It is one of the most dangerous phytopathogens for coffee plantations in terms of coffee productivity and coffee cup quality. In this review, we resume the problem of CLR in Mexico and the pathogenesis of *H. vastatrix*. The review abord plant-pathogen interactions which lead a compatible or incompatible interactions and result in CLR disease or resistance, respectively. The review abord *Coffea* spp. defense response pathways involved in *H. vastatrix* pathogenicity. Additionally, current measures to control *H. vastatrix* proliferation and germination were aborded focused on phytosanitary actions, and biological and chemical control. Finally, new trendlines to reduce the impact of CLR as nanoparticles and nanotechnology were analyzed.

KEYWORDS

Coffee leaf rust; *Coffea arabica*; pathogenesis; nanoparticles; biological control

Nomenclature

AgNPs	Silver nanoparticles
CLR	Coffee Leaf Rust
CBB	Coffee Berry Borer
CeNPs	Cerium nanoparticles
CeONPs	Cerium oxide nanoparticles
CLM	Coffee Leaf Miner
CMCS	Carboxymethyl chitosan
CSB	Coffee Stem Borers
CS	Chitosan
CuNPs	Copper nanoparticles
CuO-NPs	Copper oxide nanoparticles
Cu/Zn-NPs	Bimetallic copper/zinc nanoparticles
CWSB	Coffee White Stem Borer

DAMPs	Damage Associated Molecular Patterns
EO	Essential oil
ET	Ethylene
ETS	Effector-Triggered Susceptibility
HDT	Timor Hybrid
HR	Hypersensitive response
HMCs	Haustorial Mother Cell
ISR	Induced Systemic Responses
JA	Jasmonic acid
Ms	Mentha spicata
MnNPs	Manganese nanoparticles
NPs	Nanoparticles
OEE	Orange essential oil
PAL	Phenylalanine ammonia-lyase
PAMPs	Pathogen Associated Molecular Patterns
PbS NPs	Lead Sulfide nanoparticles
PE	Penconazole
PR	Protein-related Pathogen
PRRs	Pattern Recognition Receptors
PTI	PAMPs triggered immunity
RLKs	Receptor-Like Kinases
RLP	Receptor-Like Protein
ROS	Reactive Oxygen Species
SA	Salicylic acid
SeNPs	Selenium nanoparticles
SiNPs	Silicon nanoparticles
SNPs	Silver nanoparticles
TiNPs	Titanium nanoparticles
TiO_2	Titanium oxide nanoparticles
ZnNPs	Zinc nanoparticles
ZnO-NPs	Zinc oxide nanoparticles

1 Introduction

Coffee trade is one of the four main commodities in agricultural products, and it could be the second commodity in countries in development, especially for coffee export. Around the world, coffee commerce represents an income for over 20 million of families in 56 countries [1,2]. In 2022, Mexico was in the 7th place in *Coffea arabica* worldwide production with 702,686.02 ha dedicated to coffee planting with productivity of 1,025,034.80 tons as well as a significant income of 6,534,603.54 thousand Mexican Pesos [3,4]. In 2023, Mexico was in the 6th place of *C. arabica* worldwide production and in the 10th place of coffee in general (*C. arabica* and *Coffea canephora*) worldwide production [4]. In Mexico, the coffee commerce could significantly affect the economic dependence of 300,000 coffee producers where 96% of the cultivated surface is destined for *C. arabica* and 80% of the national production is focused on the exportation of coffee [2,5].

Coffee Leaf Rust (CLR) is caused by *Hemileia vastatrix* which belongs to the Basidiomycota phylum, Pucciniomycetes class, and Pucciniales order [6]. *H. vastatrix* uredinospores deposits in the leaf underside and is an obligate parasite, which means *H. vastatrix* feeds through on coffee leaves to live and complete their reproductive cycle while their host is alive (feeding from host nutrients). CLR disease symptomatology enlists the apparition of chlorotic spots in leaves, reduction of the potential photochemical yield of photosystems, defoliation, and reduction of coffee plants' productivity and quality of produced coffee. Also, the disease interferes with plant growth and development because of the limitation of coffee plants in nutrient uptake. Even in controlled plantations, the symptoms of diseased plants can continually evolve and affect the growth and development of coffee plants in subsequent years [7–10].

The losses in coffee productivity are divided into primary losses and second losses, the primary losses involve fruit loss by defoliation and death of damaged branches, and the second losses are caused by the cost of energy to plants to recover from the primary losses [11]. Coffee productivity losses by CLR can lie between 15%–20% and increase up to 70% [9,10]. In Colombia, losses of about 31% were reported from 2008 to 2011 in comparison with the production of 2007 [12]. In 2016, Mexico reported losses of 50% of the national production due CLR outbreak struggling the country since 2012 [13,14]. Guatemala reported losses between 59%–70% in 2012 [15]. After the crisis of 2013, Peru reported losses of up to 60% of its coffee production [16]. Centro America reported losses of up to 30% in 2013 [17]. Recent CLR outbreaks report an annual loss between one to two billion US dollars [18,19].

H. vastatrix affects domestic and wild *Coffea* spp. in the majority of *C. arabica* varieties and some *C. canephora* varieties, the CLR can be considered the most severe disease in coffee plantations due to its high propagation rate, in records CLR has reported monumental losses as was informed in Ceylon (now Sri Lanka) at the first CLR reported apparition in 1869 when the island was one of three major coffee producers worldwide; the disease spread to the main coffee producers states in Ceylon quickly with devastating consequences, and near of a decade later, it forced the coffee producers to abandon their production and dedicate to other plantations as tea plantations [20–22].

After the first report of CLF apparition, *H. vastatrix* spores spread to other coffee-producing countries. By 1990, the presence of CLR was reported in all coffee-producing countries except the island of Hawaii in the U.S. where the first apparition of CLR was reported in 2020 [7,9,20–24].

The germination of *H. vastatrix* spores is favored by dark humid environments between 21°C–27°C and available water on the host leaves for 8 h. Other factors can influence the grade of severity, e.g., the cultivated genotype determines the compatibility of the *H. vastatrix* spores and coffee plant interactions. Typica and Borbon *Coffea arabica* varietals are more susceptible to CLR disease. Also, the plantation density impacts the disease severity due to the dark-wet microenvironment generation in high-density coffee plantations. Fruit load in coffee plants also impacts the severity of the disease, in high-loaded plants the phenolic compounds with antimicrobial activity are mobilized to coffee berries [7,8,25,26].

H. vastatrix-Coffea spp. Interactions begin with the first contact of uredinospores and leaves underside. In optimal conditions and scenarios to develop a compatible interaction, uredinospores stick to epidermal cells and the germination tube grows until reaches the raised lips of the subsidiary cells of stomata [11]. *H. vastatrix* uredinospores germinates between 10°C–35°C with an optimum germination growth between 21°C–23°C, at temperatures below 15°C and above 35°C the fungal growth is slowed [19,27]. Altitude and rainfall patterns also affect the severity of the disease, in higher rainfall seasons the germination of uredinospores is favored [11]. Altitudes negatively impact the CLR severity, since CLR severity decreases with the altitude increase [28]. This is related to the decrement in the mean annual temperature in highlands in comparison with lowlands crops [28].

Pale et al. analyze the relationship between altitude, rainfall, and temperature level with CLR severity, their study analyses the data sets available by government organs in Mexico in different coffee producer municipalities. The results show a positive relationship between CLR severity and harvest period related to consistent and ripe fruit phenological stage in the coffee plants, the vegetative growth stage shows the lower CLR severity, and in the stage of fruit ripening the severity starts to increase. The results also were consistent with the positive relationship between rainfall seasons to CLR severity, rainfall seasons increase CLR severity due to the incensement in environmental moisture and leaf wetness, as well as increase the CLR incidence by water droplets splashed from infected leaves to healthy leaves [11].

CLR Disease's first symptoms show as small chlorotic spots with 1–3 mm diameter and turn to orange when the infection sorus is formed. The principal infection structures are the uredinospores which were produced at the infection sorus. As the severity increases, the uredinospore production increases at the underside leaf. *H. vastatrix* uredinospores spreads by direct contact and can be mobilized by wind or water splashes [7,8,25,27]. *H. vastatrix* uredinospores can ascend to the atmosphere and propagate to other coffee plantations by wind turbulence and rain [29].

Since coffee plantations are a complex agroecosystem, understanding how the factors at the agroecosystem interact and impact the CLR dispersion and severity can help to adapt some agronomic practices to disease control [16,29,30]. Several techniques exist to prevent *H. vastatrix* germination and control CLR disease, e.g., proper crop management can be adapted to the current agricultural practices at the coffee plantations [31].

For the chemical part, the producers can apply some foliar fungicides and is recommended to apply them at the vegetative growth stage when the CLR incidence is still low [11]. The protective fungicides usually are made of copper compound and generate a protective coating in the leaves, these fungicides just prevent the first infection stages of *H. vastatrix* and slow down their propagation. On the other hand, systemic fungicides usually compounded by triazoles are more aggressive than protective fungicides, systemic fungicides inhibit ergosterol production [8,32].

Another chemical treatment alternative is the emerging field of nanotechnology. Nanotechnology is based on the synthesis or modification of nanoscale materials [33]. Nanoscale materials as nanoparticles can be synthesized from two main approaches, bottom-up and top-down [33]. Among the main advantages of the use of nanotechnology is the easy surface modification of nanoparticles to specific functions or applications [34]. Nanotechnology has gained attention in several agriculture fields due the its potential application in food safety packages, crop productivity, phytopathogens control, and soil health [33,35,36].

The present review provides an overview of our current knowledge of the coffee-*H. testatrix* interaction, mainly regarding pathogen infection and variability, and multilayered host defense responses. This review also contains information about the main phytosanitary measures to control coffee H. *vastatrix* and the potential use of nanoparticles as a possible control of this pathogen.

2 *Hemileia vastatrix* Diseases

The Pucciniales (before named Uredinales) is a fungal order phytopathogen commonly known as Rusts. The Pucciniales represent one of the major severity and complex phytopathogens. The Pucciniales order is composed of 120 genera and 6,000 species. The main affected agriculture products are wheat, soja, and coffee crops which represent a high economic impact derived from crop losses [37]. Pucciniales are known for having a constant evolution with their host, which resulted in their adaptability and resistance. The Pucciniales infect their host as obligate biotrophic pathogens feed from the host cells' nutrients, which means the pathogen feeds and grows only if the host is alive. In other words, rust depends on a living host to grow and reproduce [38]. Focused on *H. vastatrix*, their co-evolution with their host makes it more difficult to generate coffee resistance speciesn [39,40].

The rusts are categorized as specific host pathogens and their reproductive cycles are differentiated by their sporulation stages and how complete their reproductive cycle [39,41]. The rusts are caused by biotrophic fungi, and depend entirely on their living hosts, some rusts need two taxonomically different hosts (aecial and telial hosts) to complete their reproductive cycle (heteroecious), other rust (autoecious) as *Hemileia vastatrix* only have one kwon specific host and can complete their reproductive cycle above the same host or in different plant from the same species [23,37,42].

Fungi-caused rusts share some properties as they have highly developed infection structures, one of them is the haustorium a specialized structure to makes possible the nutrient uptake from the living cell host. Haustorium derives from *haurire* (in Latin), which means to drink or to draw [38]. Besides the haustorium role in nutrient uptake, they have limited secretory activity related to their lytic enzyme's secretions and ensure the plant-pathogen compatible interaction due to the effectors delivery which suppresses the host defense signals for long periods and have a developed defense structure that separates the fungal and plant plasma membranes [23,43,44].

H. vastatrix is classified as primitive rust, since CLR discovery has a diverse catastrophe registered, the first registered apparition of *H. vastatrix* was in the Ceylon (now Sri Lanka) epidemic in 1869 [20–22,40]. It is a biotrophic pathogen and, hence needs a live host to live, feed, and complete its reproductive cycle. Besides *C. arabica* being susceptible to two rusts caused by *H. vastatrix* and *H. coffeicola*, only *H. vastatrix* has an economic worldwide impact on coffee cultivars countries [40].

H. vastatrix has ovoid/reniform uredinospores (single cell) with protuberances/spines at the dorsal and convex face and smooth surfaces in the ventral and concave face, uredinospores have thick walls and hydrophobic surfaces (Fig. 1). Mature uredinospores can develop up to 300 spines on average at their surfaces, their spines are grouped and functioned to grip and hold on into foliar tissues [42]. *H. vastatrix* teliospore has a smooth surface and its uredinospores and teliospores have hyaline walls with 1 um thickness. *H. vastatrix* forms supra stomatal sori with bouquet shapes [40].

The life cycle of *H. vastatrix* is hemicycle with autoecious reproduction. When the uredinospores achieve the invasion of coffee leaves, start to produce reproductive structures such as uredinospores, teliospores, and basidiospores. Uredinospores are the principal inoculum source to spread *H. vastatrix*. Basidiospores cannot penetrate and infect coffee leaves and teliospores rarely reinfect their host [40,42].

H. vastatrix uredinospores resist harsh environmental conditions and can survive in long drought periods or nutrient shortages and low temperatures. Uredinospores can stay dormant until the environmental conditions change. Uredinospore germination requires free-water, dark-wet environments for periods of 6–8 h. Environments between 21°C–27°C with high relative humidity (up to 80%) improve uredinospores gemination. Uredinospores were renewing at the infection sorus and represent the most important infection structure for the asexual cycle of *H. vastatrix* [6,19,39,40,42,45].

At the end of colonization, mature uredinospores develop infection sori and generate new uredinospores, basidiospores, and teliospores [24]. Teliospores produced promycelium which produced basidiospores [40].

The infection by *H. vastatrix* begins at the penetration phase by the uredinospore's adhesion to the underside leaf surface, a crucial step in the *H. vastatrix* pathogenesis because it prevents the pathogen displacement from their host and improves the thigmotropism. Uredinospores-host first contact is fundamental to the establishment of the signal chain via transmembrane proteins [42,46]. By signal chain activation *H. vastatrix* can receive and process extracellular information into transcription factors, activating protein synthesis pathways to maintain the cellular wall thickness and morphogenesis [42,46].

After the first contact, the germination tube is formed during the uredinospores germination in the dikaryotic phase, the germination tube end has a hypha hook shape tip which senses and transduces the leaf topography information via signal chain, and the appressorium is formed after the stomata identification [23].

The appressorium is the first structure formed above the stomata by the uredinospores germination; this makes the stomata penetration begin. During the appressorium formation, lipids and glycogen are transferred from the cell to the appressorium. Once it is over the stomata, a penetration hypha grows to penetrate the stomata and reach the substomatal chamber with the differentiation of the substomatal vesicle [23,40]. In the parasitic phase, the penetration hypha differentiates to produce lateral branches that subsequently differentiate into haustorial mother cells (HMC) to produce a haustorium after its first contact with the mesophyll cell wall. Each lateral hypha resembles an anchor [23,40,42]. After 36 h of inoculation, the first haustorium is formed, infecting the near subsidiary cells [23,40].

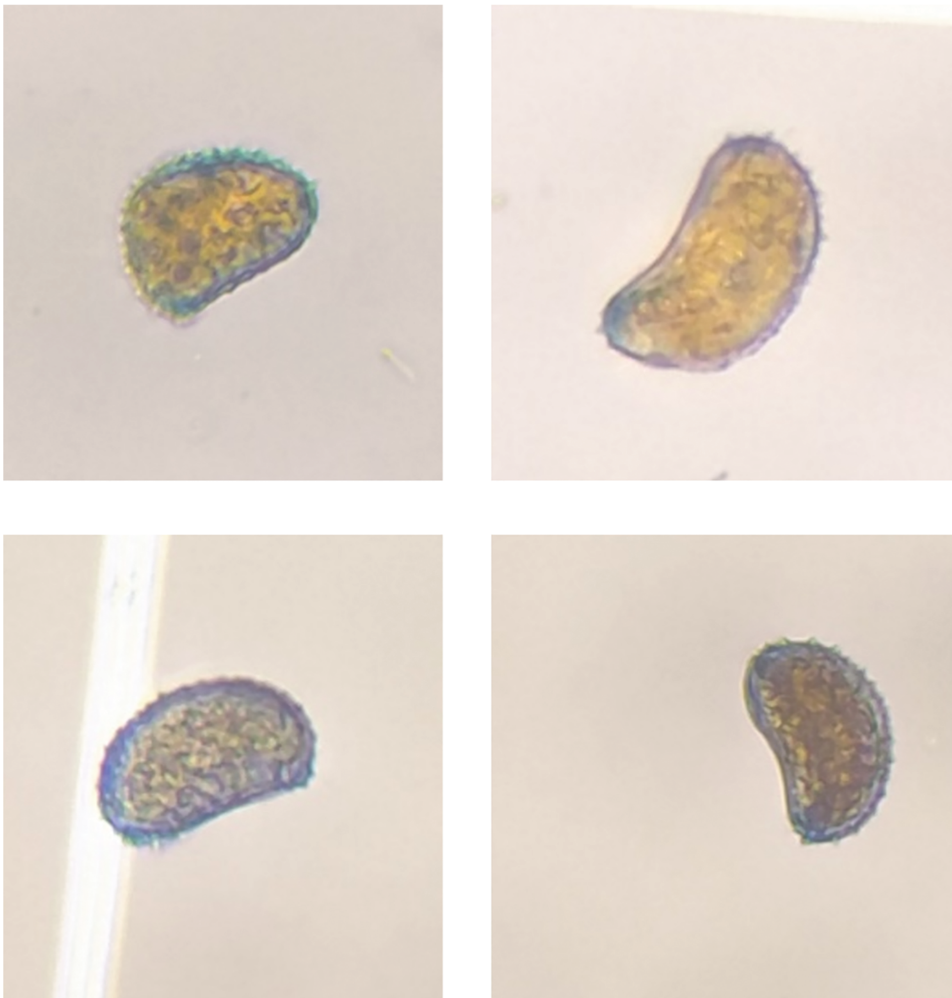

Figure 1: *Hemileia vastatrix* uredinospores isolated from a coffee plantation in Angel Albino Corzo, Chiapas, Mexico with coordinates 15°45′33.8″N 92°38′03.3″W

As pathogenesis progresses (Fig. 2), *H. vastatrix* continues to form intercellular hyphae, HMCs, and haustorium, to invade substomatal cavities such as lower epidermal cells, mesophyll, spongy and palisade cells. At this stage, a dense mycelium is formed under the penetration stomata; at this point, chlorotic spots may be visible [23,40,47]. 21 days left, the sporulation phase begins with uredinospores bouquet shape sori are formed through the stomata as suprastomatal sori (Fig. 3A), at this stage, the chlorotic spot turns orange (Fig. 3B) and the uredinospores sori produces new infection structures which can be propagate easily through wind, rain splashes, and insects to restart the cycle in other leaves or plants. In advance of the disease severity, new uredinospores sorus are produced in the same leaf and the near leaves (Fig. 3C) [23,38,40,42,47].

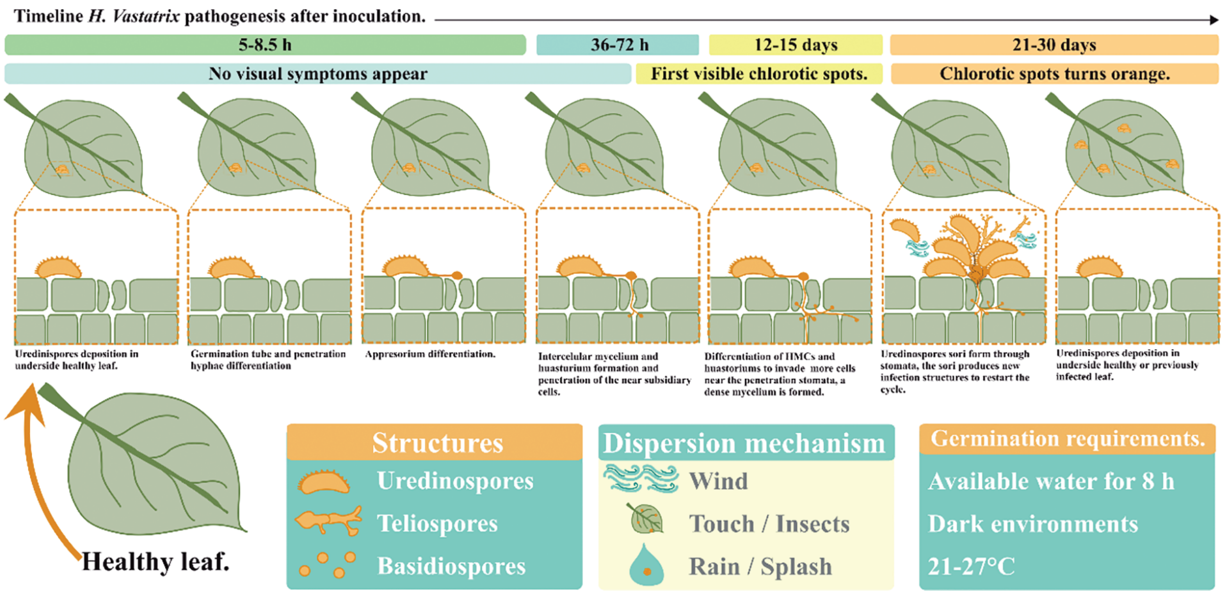

Figure 2: *Hemileia vastatrix* reproductive cycle [23,40,47]

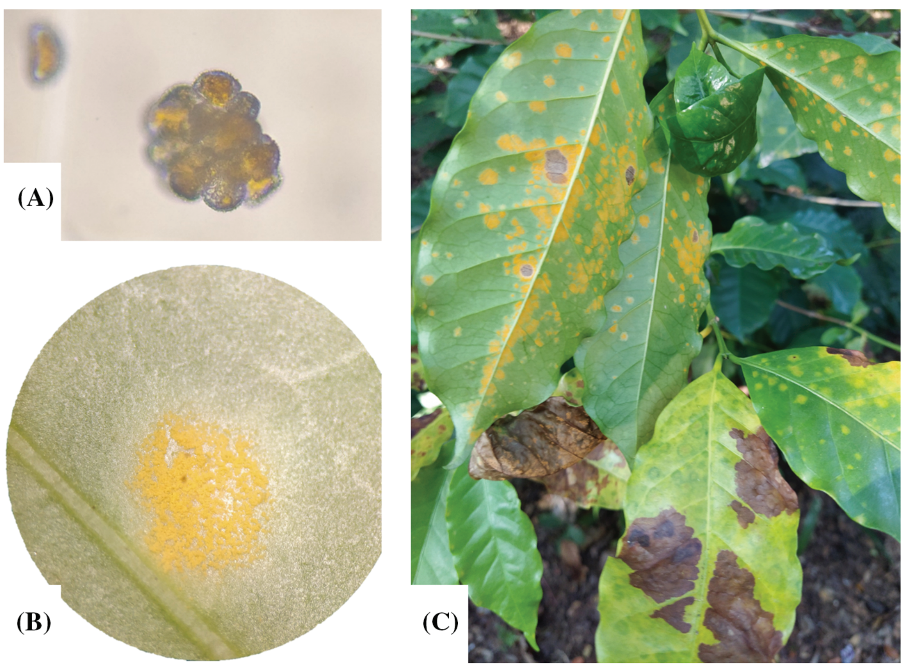

Figure 3: *Hemileia vastatrix* uredinospores and Sorus viewed in stereoscope (A) the uredinospores were isolated from coffee leaves with CLR (B) in a coffee plantation in Angel Albino Corzo, Chiapas, Mexico with coordinates 15°45′33.8″N 92°38′03.3″W (C)

3 *Hemileia vastatrix-Coffea* spp. Interactions

Rust fungi's reproductive cycle usually involves two parasitic stages, dikaryotic and monokaryotic. The monokaryotic phase is related to basidiospore germination, basidiospores penetrates directly the epidermal cells. The dikaryotic phase starts with uredinospores germ tube formation and elongation for surface recognition. When the germination recognizes the subsidiary cells of the stomata, forms the appressorium over the stomata. The rust fungi produce sophisticated infection structures well differentiated and divided by septum, these structures help the fungi to avoid host recognition and improve the pathogenesis [38,39,43,48].

The CLR disease development starts in the dikaryotic phase when uredinospores begin in contact with the leaf's underside. For this phase the adhesion of the fungi to the leaf surface is crucial. The adhesion of uredinospores is improved by the hydrophobic nature of uredinospores and the cuticle wax layer in the host leaves. The spines at the uredinospores surfaces also improve the adhesion by mechanical grip. After the first contact, an extracellular matrix is formed with low molecular weight carbohydrates and glycosylated polypeptides. The formation of the adhesion pad seems to be by the activity of cutinases and esterases [42]. The germ tube recognizes the topography of the ridge stomatal lips and the leaf alcohols and sends the signals to start the appressorium differentiation from the germ tube, the surface recognition involves chemical and physical signals as topography, hydrophobicity, and the presence of cutin monomers [39,42,43,48,49].

The success of infection may be determined by the susceptibility of the host to the rust race. The differentiation of the infection hypha begins with one of the fungi's defense mechanisms as the production of suppressors of defense to invade their host easily [39,42,48]. Independent of the plant-pathogen interaction results the *H. vastatrix* uredinospores and their complex; pre and post-penetration structures have an arrangement to evade the host chitinases, where the chitin is inside the structures and the outside in contact with the host environment are labels of β-1,3-glucans [49].

The morphological differentiation due to the formation of infection hypha is crucial in the pathogenesis successful. The penetration hypha enables nutrient uptake, otherwise, the growth of the germination tube depletes the fungi's nutrients. After the first haustorium formation, the nutrient uptake is limited, hence, the fungi are forced to change their fungal wall composition. This event marks a change in protein synthesis, ribosome synthesis, and secretion of hydrolases [39,42,48].

Once the appressorium is formed above the stomata, an infected peg starts to grow from Haustorial Mother Cells (HMC). The infection peg grows between guard cells through substomatal space and forms the substomatal vesicle. After then the intercellular hypha is formed, the hosts start to detect the phytopathogen presence by the delivered elicitors. At the first contact of the HMC with mesophyll cells, the HMC starts to differentiate into haustorium to begin the host cell infection. The mechanisms of haustorium infection are similar to those of the appressorium, the HMC attaches to the host cell wall and forms a penetration hypha, the HMCs are protected with a thick multilayer wall, the mechanisms of appressorium penetration are related to the application of pressure and secretion of lytic enzymes over the penetration zone previously, cell wall degradation is restricted to the penetration zone [39,42,43,48,49].

The haustorium is involved in intimate communication with the host cells, as well are implicated in the suppression of host defense response and nutrient uptake [42]. This intimate communication between the haustorium and the cell hosts is possible due to the formation of an extra haustorial matrix and membrane which divides the haustorial plasma membrane and the haustorium body from the plant plasma membrane [42]. The extra haustorial matrix is composed primarily of biomolecules derived from the cell hosts and provided by the fungi during the infection, this matrix makes an intimate contact zone where the haustorium body is protected from the plant plasma [39,42,48].

The haustorium (Fig. 4) is formed from the neckband that maintains and connects the HMC with the haustorium. The neckband protects the haustorium by sealing the haustorium interfaces from the apoplast flux. The neckband is the first infection structure formed after HMC hypha penetration. The penetration hypha broke the cell host membrane due to the applied pressure and the secretion of lytic enzymes. After the haustorium formation, the extra haustorial matrix and membrane were formed. The absence of ATPase activity at the haustorial membrane implies an influx of solutes from the host cell without control [42]. The formation of the haustorial involves a re-organization of the cell host components and cytoskeleton, and a continued exchange and secretion of proteins, effectors, and elicitors from the haustorium [42,43].

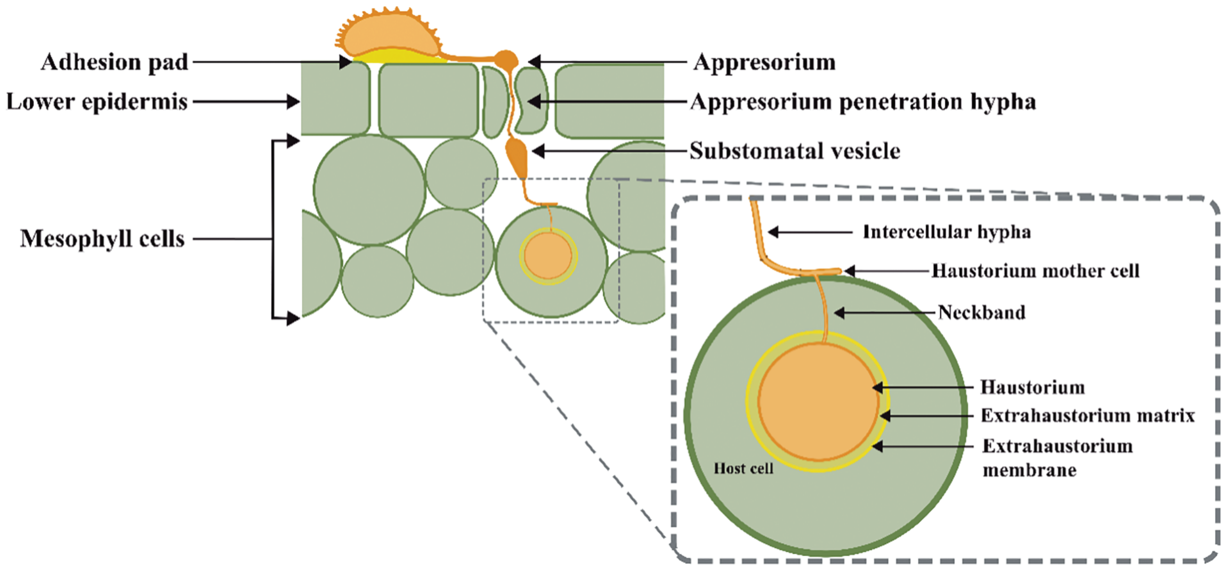

Figure 4: Schematic representation of *Hemileia vastatrix* infection structures in *Coffea* spp. leaves [42,43]

During the penetration, parasitic, and sporulation phases the fungi produce sophisticated highly specialized structures that produce proteins, enzymes, and secondary metabolites to adapt and evade the host defense mechanism and assure their sporulation phase and therefore, complete the reproductive cycle [38,42,43,50,51]. The secondary metabolite productions can alter the hormone pathways as auxins, gibberellic acids, jasmonic acid, and salicylic acid [43].

4 Coffea Defense Mechanisms

Plants' susceptibility and resistance to pathogens is determined by the interactions between host plants and pathogens. Plants have their own recognition and defense mechanisms to identify and protect them from potential pathogens and dangerous conditions. Plant defense mechanisms are complex and involve passive and active defense lines. The passive (natural) or nonhost resistance mechanisms involved some barriers to prevent adhesion and penetration of infection structures. Whitin's passive mechanism can be found in the deposition of wax cuticles, lignification of cell walls, the production of secondary metabolites, and silica accumulation [24,52–54].

The active defense lines involve the pathogen and damage recognition. The Pattern Recognition Receptors (PRRs) detect molecules related to pathogen invasion as Pathogen Associated Molecular Patterns (MAMPs or PAMPs) or related to host stress/damage as Damage Associated Molecular Patterns (DAMPs). PRRs are transmembrane proteins, in the presence of PAMPs or DAMPs that bind or release to a protein system to activate the downstream of immune signaling. PRRs detect microbial elicitors produced during the pathogen invasion (MAMPs or PAMPs), this recognition process is called PAMP-triggered immunity (PTI). PRRs are mainly receptor-like proteins (RLP), and receptor-like kinases (RLKs) [40,49,53,55].

Elicitors can be sourced from pathogen secretions (exogenous) or delivered molecules from wound plant tissues (endogenous) [49,55]. The elicitors can be specific or non-race specific. Usually, the elicitors are composed of peptides, proteins (esterases), glycoproteins, fatty acids derivatives, sterols, oligosaccharides, or polysaccharides [46,49,55]. Endogenous elicitors are linked to DAMPs and are produced in wound tissue by enzymatic lytic activity, blocking or detriment of mechanical barriers due to the advance of the disease [46,49,55].

DAMPs act as a long-distance immune mechanism and activate the local and systemic defense signaling. DAMPs are molecules released by damaged cells due to pathogen invasion or by cells being under attack. DAMP molecules are primarily fragments of polysaccharides cut off cell membranes, cytosolic proteins, and other macromolecules as peptides, amino acids, and nucleotides [53]. PAMPs and DAMPs can activate immune responses to PTI in synchrony to increase or amplify the immune signals, as well PTI and ETI can overlap to work in parallel or in synchrony to suppress the pathogen propagation [24,53].

Effector Immunity (ETI) is another active defense mechanism based on the pathogen invasion detection by pathogen effectors. Effectors are small proteins produced and mainly secreted by pathogens to achieve colonization [55–57]. Effectors can modify the structure and function of host cells and also can modify the host metabolism and defense pathways. Some effectors are capable of interfering with PTI and ETI which results in a compatible interaction due to the induced Effector-Triggered Susceptibility (ETS) to the host [24,55–58].

When the effector is detected by R protein receptors active ETI or PTI the invaded plant starts to alert the defense system. Hence the host cells activate a signal cascade related to increased ion fluxes. The increment of cytoplasmatic calcium concentrations is related to PTI. Calcium ions function as a secondary messenger for the production of stress-related hormones. This activates the signaling pathways of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). The SA pathway is the more related pathway to suppress the compatibility interaction due to the activation of defense enzyme mechanisms. SA activates the expression of phenylalanine ammonia-lyase (PAL), oxidative burst, ROS accumulation, and protein kinase cascade activation [24,32,55,56]. In coffee-resistant cultivars, the SA pathway is activated by the expression of resistance genes (*CaNPR1, CaPR1*, and *CaPR5*) [59]. ETI and PTI can induce similar defense mechanisms, e.g., both can activate the salicylic acid pathway considered as the main defense mechanism against *H. vastatrix*, but ETI responses seem to be more prolonged than PTI [24,55,56].

Other defense mechanisms involved in *Coffea* spp. response against *H. vastatrix* is the activity of the enzyme Polyphenol Oxidase (PPO), an enzyme containing copper as a cofactor and 5-caffeolyquinic acid (chlorogenic acid) as a substrate. PPO catalyzes the oxidation of phenolic compounds (monophenols → *o*-diphenols → quinones) near to wound site when the tissue is damaged stopping the germination by Hypersensitive Response (HR) acceleration, reducing the bioavailability of alkylate proteins and by the improvement of polymerization of proteins to form physical barriers [60–63]. Other post-haustorial defense mechanisms are related to the enzymatic activity of Plant Peroxidases (PODs) as PPO, PAL, β-1,3-glucanase, chitinase by the degradation of chitin at the *H. vastatrix* cell wall, and lipoxygenase [32,63–65].

Pathogens can respond to plant defense systems to avoid it and lead to a successful pathogenesis. In the same way, plants can prevent or respond on time to the pathogen invasion and break the pathogenesis. Therefore, leading a successful resistance to that pathogen. E.g., the HR activation by the expression of the gene CaNDR1b in coffee cultivars is involved in pathogen recognition [59]. The HR is activated with the first haustorium formation as an early defense mechanism by the detection of effectors by PRRs. This provokes the production of Reactive Oxygen Species (ROS) to "eliminate" the infected cell and cut the pathogen nutrient uptake. The activation of HR also is related to the deposition of callose, cell wall lignification, and synthesis of chlorogenic acid [49,51,53,58,66,67].

These responses are the result of the co-evolution of some plant pathogens and could provide some physical or chemical barriers and the production of elicitors or effectors to activate or deactivate the barriers of the counterpart [49]. In dependence of the organism's genetic material, these barriers or defense mechanisms could be activated as preventive and protective mechanisms and others could be induced by the detection of pathogen attack. Some defense mechanisms are hypersensitive reaction, phytoalexins production, structural barriers, production of elicitors, and activation of systemic mechanisms [42,49–51].

H. vastatrix interactions with *Coffea* spp. respond to the gene-for-gene model proposed by Flor as *Coffea spp*. race-specific defense mechanism [68,69]. The gene-for-gene model explains how plant-pathogen interactions could lead to susceptibility or resistance because of specific parasite responses, the model explains the resistance when each elicitor produced by the virulence gene expression and interacts with a virulence gene receptor in the host organisms, this binding active a signal cascade and therefore plant defense system and produced an incompatible plant-pathogen interaction [55]. Each effector expressed by a specific *Avr* gene is recognized by a specific *R* gene, leading to an incompatible interaction. When the host and pathogen have a straight co-evolution relationship, the *Avr* genes tend to mutate to evade the recognition system by the host R genes leading to a compatible plant-pathogen interaction or a susceptibility case of the host to the pathogen [55,58,67,70].

In the gene-for-gene model, each dominant resistance gene (S_H1-S_H9) conferring coffee leaf rust resistance for *coffee* spp. is one virulence gene (v1-v9) in the *H. vastatrix* genome, and the genes are present individually or in groups for each *Coffea* species or *H. vastatrix* race [13,49,68–70]. The Severity of the pathogenesis is determined by the interaction of the genes in the *Coffea* species and *H. vastatrix* race. The recognition of *H. vastatrix* virulence genes by resistance genes in *Coffea* species tends to an incompatible plant-pathogen interaction. The v1-v9 genes' presence in *H. vastatrix* races defines their severity, at now at least 50 *H. vastatrix* races are known worldwide, and the races with less genetic material are more dangerous as their sporulation times and capacity are shorter than the races with more virulence genes [13,49,56,68,70,71].

In Mexico and Brazil as in other coffee producers in Central and South America race II (v5) is the most common in coffee crops. In America races I (v2, v5), III (v1, v5), XV (v4, v5) and X (v1, v4, v5) are also common [13,72]. In pure arabicas are found genes S_H1, S_H2, S_H4, and S_H5, in *C. liberica* is found the gene S_H3, in *C. canephora*, are found the genes S_H6, S_H7, S_H8, and S_H9, and also found in Timor Hybrid HDT (S_H6-S_H9) due its hybridization origin from *C. arabica* × *C. canephora* [13,69]. *Coffea* genotypes can be classified according to their resistance or susceptibility to *H. vastatrix* races, Group A is related to *coffea* genotypes with resistance to all known *H. vastatrix* races, while Group E is for the *Coffea* genotypes with susceptibility to all known *H. vastatrix* races [49,69].

Following the concepts in the gene-for-gene model, *Coffea* plants can perform a complete or partial resistance to CLR; complete resistance is achieved when the defense S_H1-9 genes bind with virulence v1-9 genes in *H. vastatrix*. Incompatible plant-pathogen interaction is expressed in the resistance *coffea* genotype when homozygous dominant genes AA and RR are present in each organism (*H. vastatrix* and *Coffea* species respectively), dominant heterozygous Aa and Rr also result in an incompatible plant-pathogen interaction, while recessive genes aa and rr results in a compatible plant-pathogen interaction or a successful disease [46].

Plant-pathogen interaction between *Coffea* spp. and *H. vastatrix* leads to a compatible, incompatible, and partially compatible, thus as a result the balance of *Coffea spp*. defense mechanisms expressed and *H. vastatrix* adaptability and pathogenicity (Fig. 5). As mentioned above, *Coffea* spp. have different defense mechanisms involving their natural or passive and active mechanisms related to a specific host or no, and these mechanisms can be classified as those expressed before the first haustorial formation and those expressed after the first haustorial formation [24].

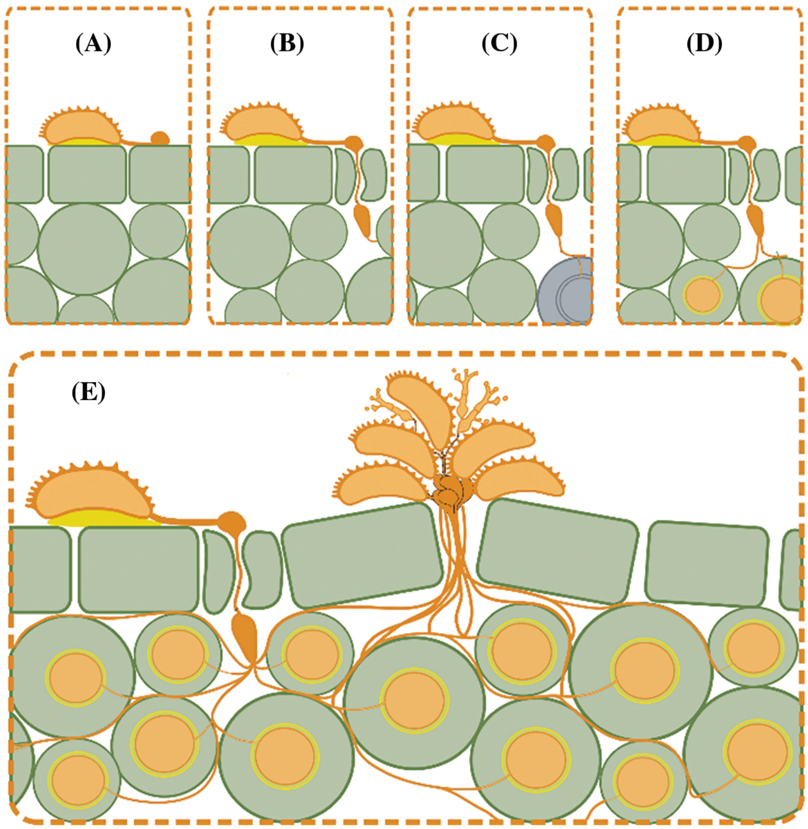

Figure 5: Schematic representation of possible plant-pathogen interaction scenarios where (A) represents unsuccessful interaction due specificity of appressorium hypha to penetrate stomas as represented in (B). (C) represents an incompatible interaction due hypersensitive response of *Coffea* sp. due pathogen recognition system, which is avoided in (D) by the effectors liberation by haustorium leading in a full compatible interaction as is represented in (E) [42,43]

HR in *Coffea* species is one of the main defense responses and is activated in both cases, compatible and incompatible interaction, HR can be activated and amplified by PTI and ETI at the first contact with the uredinospores with host leaves, but the ETI mechanism is more related with a complete incompatibility interaction or disease resistant [24]. HR usually begins in stomata and subsidiary cells as guard cells and mesophyll cells [24]. Other effective defense mechanisms in incompatible interactions are associated with pathogenesis-related proteins (PRs) as PR2, PR3, PR10, PR15, and PR16 were related to incompatible responses, especially PR2 (β-1,3-glucanase) and PR3 (chitinase) [24,64].

Luján-Hidalgo et al. [73] reports in 2020 the biochemical responses of *C. arabica* plants to *H. vastratrix* infection where they found a significant increase in the amount of caffeine and gallic acid presence in infected leaves in comparison with uninfected ones which results can be related to Induced Systemic Responses (ISR), as well an increase of enzymatic activity (chitinases and β-1,3-glucanases) associated to protein-related pathogen (PR) [64,73]. An emerging alternative in the control of CLR is the technological development from the genomics perspective, e.g., the foliar application of RNA interference (RNAi) using spray-induced gene silencing (SIGS). SIGS can be applied to silence pre and post-exhaustorial effectors genes in *H. vastatrix* uredinospores germination [74].

5 Current Control of Coffee Leaf Rust (*Hemileia vastatrix*) in Coffee Plantations

The main phytosanitary measures to control CLR propagation are preventive and corrective actions, in the preventive action is the control of plant population density to prevent shadow excess by the overlapping of coffee plants. In some cases, the farmers tend to breed or cultivate CLR-resistant cultivars such as HDT, a resistant cultivar derived from the natural hybridization of *Coffea arabic*a × *Coffea canephora* with the resistance genes S_H6-S_H9 [6,75]. In general, the main actions to control CLR can be divided into phytosanitary measures where the farmers take actions to prevent favorable germination and sporulation conditions of *H. vastatrix*, where the flowering cycles can be used as an indicator to start the phytosanitary actions [76]. Genetic control with resistance cultivars and breeding with new resistance cultivars by resistance genes present in Timor Hybrid HDT [6,75].

The management of shadow and pruning helps in the control of the disease, excess of shadow reduces the photosynthetic reactions in the lower coffee plants by the canopy, also, the overlap of branches in the canopy generates microclimates and promotes the germination of *H. vastatrix* spores [16,31]. Pruning as a renewal technique removes the old unproductivity branches, which could be a source of inoculum for several microorganisms, and promotes aeration and photosynthesis [16,31].

Gonzales et al. demonstrate the relationship between shadow system, pruning techniques, and cultivated genotype in a study conducted in 2022. The study analyzes the effect of pruning techniques (non-pruning, descope, medium pruning, and recipe) in susceptible *C. arabica* varieties such as Typica and Pache. Their results reduce de percentage of incidences of CLR from non-pruning by 37.81% in contrast with coffee plants pruning by recipe method by 16.15%. The reduction in the percentage of incidence was more representative in *C. arabica* Typica than in Pache varietal in the difference shadow system and pruning methods analyzed [16].

Reducing the plantation density also can reduce the CLR severity, in high-density plantations the environmental moisture between plants and the plant-to-plant interactions can be increased [29]. A studio simulated by Mora et al. analyses the interplay between harvesting, plant density, and ripening time in the dispersion of CLR. The results show an increment in CLR dispersion in high-density plantations analyzed from 500 plants/ha to 5000 plants/ha, where the rust incidence increases from 500 to 3000 plants/ha with the highest incidence at 2000 plants/ha. The study also shows the importance of caring about synchrony in the ripening, asynchrony ripening can lead the agricultures to work long distances per day, which increases the probability of spreading the uredinospores by contact [29].

Coffee plantations shadowed by trees also play a crucial role in CLR dispersion, planted trees in the agroforestry systems can improve the coffee plants metabolism and the physiological resistance [30,77]. The shape of trees at the canopy can increase the CLR severity by the microclimate generation and reduce the CLR dispersion. Planted trees at the periphery can reduce the CLR dispersion airborne [16,29,30]. In a study conducted by Daba et al. in 2015/16 and 2020/21 showed lower CLR incidence in a forest coffee system (FC) than in a semi-forest coffee system (SFC), in FC the human activity is limited so the dispersion of urediniospores is limited, also the dense canopy in the FC reduces the wind speed and penetration of water droplets [77].

Chemical control involves the use of organic or inorganic pesticides. Biological control involves the use of natural pesticides elaborated with plant extracts or by the application of antagonist microorganisms [41]. Chemical and biological control have special attention due to their function of elicitors with properties to active natural defense pathways in coffee plants. For, Lam et al. reported in 2019 the effectivity of methanolic extract of *Baccharis glutinosa* roots in *C. arabica* Var. Borbon and Caturra with significative inhibition of *H. vastatrix* uredinospores germination [41]. The results show a significant decrement in the germination capacity of uredinospores from 38.54 to 7.01, 1.67, and 2.2 in leaves treated with copper oxychloride and MEBs 100, 270, and 750 mg mL^{-1}, respectively [41].

Ramirez et al. reported in 2020 a study conducted in 2018 in Oaxaca, Mexico. The study evaluates the CLR severity in *C. arabica* var. Geisha seedlings inoculated with *H. vastatrix* uredinospores. They compared the effect of 24 treatments by foliar application to Geisha seedlings. The treatments with the higher inhibition were Bacit-Sur+Nat-R composed of colonies of *Bacillus subtilis* and Trichoderma spp. with hepar sulfur at 15%, *Bovista plumbea at* 5% and homeopathic plant extract at 10% in water. The treatment Bacit-Sur+Nat-R shows a significative lower CLR incidence in contras of copper oxychloride (Oc) [10].

Besides the agronomic phytosanitary measures, the application of chemical products can lead to protecting coffee plants in a preventive or systemic way. Cupric pesticides usually are used in combination with systemic or systemic pesticides to avoid pathogen resistance to pesticides [78,79]. Cupric fungicides are the most used as protective or contact fungicides due to their capacity to make a coating over the treated leaves. Furthermore, copper improves the vigor and retention of coffee leaves [80]. The availability of copper ions in the foliar surfaces inhibits the first stages of infection of *H. vastatrix*.

If the used pesticide contains copper in its reactive chemical formula (Cu^{2+}) the copper ions can reduce the germination of urediniospores and penetration of hyphae because copper inhibits the respiratory process, and protein synthesis and weakens the cell membrane, also the copper tends to interact with pathogen cell wall due their affinity to carboxyl groups available on the surface [6,8,31,79–81]. Cu ions can cause cell death by their capability to interrupt metabolic pathways, development of oxidative stress, and loss of membrane integrity due to Cu ions absorption which leads to the generation and accumulation of ROS [80,81].

Inside the main disadvantage of copper fungicides is the need to reapply constantly to protect new leaves and due their washability by rain or irrigation. Also, the copper fungicides start to lose efficiency at 10% of infection of *H. vastatrix* [6,8]. The main used copper fungicides are copper oxychloride, copper hydroxide, copper oxide, and copper sulfate [6,8,19].

The corrective or systemic fungicides can stop the infection in the early stages (before sporulation), in comparison with protective pesticides, the systemic pesticides are absorbed by the plant and transported to act on the infection site, in other words, the systemic pesticides work from inside the leaves instead the leaves surfaces. E.g., the triazole compounds inhibit ergosterol production but the effectiveness is reduced at the sporulation stage of the disease. Mesostemic fungicides as strobilurins work from the wax cuticle in the leaves and inhibit the sporulation of *H. vastatrix*. Hence is recommended the combined application of strobilurins and triazole compounds [6,8,78,79,82]. The mixture of triazoles and strobilurins can be applied in the soil or the foliar surface [78,79,82]. Some common triazoles used in Mexico and Latin America are epoxiconazole, cyproconazole, azoxystrobin, and tebuconazole which can be applied alone or in combination [83].

6 Potential Use of Nanoparticles in the Control of Pests and Diseases in Agriculture

The nanoparticles (NPs) can be defined as a particle with a length of 1 to 100 nm in any axis (x, y, or z), at this dimension the particles behave as "big molecules" instead of bulk materials. This improves the reactivity and the surface area making it more effective in the nanoparticulate form than in the bulk size [84,85]. The nanoparticles can be classified mainly by the precursor material or by the synthesis mechanisms. When the nanoparticles are obtained from a macroscopic source the synthesis route is called "top-down" and the mostly methods used are electrochemistry, nanolithography, laser ablation, and thermal decomposition [84,86,87]. The route is called "bottom-up" when the nanoparticle synthesis starts from chemical precursors as in sol-gel, photosynthesis, chemical reduction, and nano-emulsions among others. Otherwise, can be classified in order of their chemical source as organic or inorganic nanoparticles [84,86–88].

The application of nanoparticles has been popularized due to the surface-area ratio improving the efficacy over their bulk-size counterparts. The nanoparticles have been studied and applied in the agricultural industry in the development of technologies for food packaging, pesticides, and fertilizers. In these formulations, the nanoparticles are considered as an active ingredient or as a vehicle for active ingredients. The main studied inorganic nanoparticles in the control of phytopathogens are the nanoparticles from silver (AgNPs), copper (CuNPs), zinc (ZnNPs), Cerium (CeNPs), silicon (SiNPs), and titanium (TiNPs) [88–90].

Organic nanoparticles also have been applied in the control of phytopathogens, e.g., the application of salicylic acid and chitosan nanoparticles in the control of *Punccinia triticina*, the nanosystem report to causes the lysis of the uredinospores [91]. The functional groups in the surfaces of the nanoparticles can be modified to functionalize the nanoparticles or to synthesize a nanocomposite or a nanosystem [88–90]. SiNPs and TiNPs are commonly functionalized to use as vehicles for active compounds. E.g., the surface modification of SiNPs with chitosan oligosaccharides tested *in-vitro* in the inhibition growth of *Fusarium* spp. [90].

Recently has been reported the application of AgNPs and CuNPs in the suppression of germination of *H. vastatrix* uredinospores [92]. Little information has been reported on the application of nanotechnology in the control CLR or in the inhibition of germination of *H. vastatrix* uredinospores. Table 1 resumes recent publication information on the control of phytopathogens by nanotechnology.

The main action mechanisms of nanoparticles involve the generation of ROS by the photocatalytic activity of nanoparticles, thus damaging the cell organelles, and biomolecules as oxidation of proteins and DNA degradation, inducing oxidative stress and interfering with the electron chain transport [107,108]. The concentration of produced ROS depends on the nanoparticle's chemical nature and nanoparticle dose [107]. Metallic nanoparticles can release metal ions, the released ions can modify the structure of biomolecules by their interaction with thiol, amino, and carboxyl functional groups [108]. In photosynthesized nanoparticles, the released ions can carry antimicrobial compounds derived from the plant extract [108].

Nanoparticles interact with the whole environment after they apply to a plant, interacting with the plant of interest, the phytopathogens, the soil, and the habitats of the soil [109,110]. The nanoparticles can enter the plant depending on the application via the nanoparticle physicochemical properties [109]. Nanoparticles can enter to plant by leaves and root uptake [109]. Can be administrated in seed imbibition or seedlings/plants by foliar application or soil supply [111]. The uptake and assimilation of nanoparticles are carried out by roots and leaf tissues such as leaf epidermis and stomata, as well as by wounded tissues [109,111]. The applied nanoparticles can mobilize or translocate inside plants by the apoplastic and symplastic pathways [109,111].

Besides the positive effects of nanoparticles in the control of phytopathogens and promoting tolerance to biotic and abiotic stress, nanoparticles can be harmful to studied plants [111,112]. The main factors in the toxicity of nanoparticles are the size, morphology, chemical nature, and dose [110,111,113]. Penetration and translocation of nanoparticles inside the plants concern the uses of nanoparticles. Thus, can generate the bioaccumulation of nanoparticles in plant organelles such as vacuoles and vesicles [114,115].

The excessive generation of ROS conduces to an imbalance with the plant antioxidant system and leads to phytotoxic effects. The excess of ROS can lead to oxidative burst, cell death, and stomata closure, leading to a reduction in CO_2 fixation [111,113,116]. The foliar application of nanoparticles in high concentrations can induce physiological damage as a reduction of the photosynthesis rate. This is related to stomatal closure and blockage of chloroplast and mitochondria electron transport chain [111,113]. Table 2 shows some phytotoxic effects of nanoparticles.

Table 1: Reported nanoparticles with inhibition growth of phytopathogens

Nanoparticle	Morphology	Size particle	Zeta potential	Synthesis route	Phytopathogen	Disease	Phytopathogen host	Application	Ref.
CuNPs	Spherical	48.07 nm	−26 mV	Biosynthesis	*Tribolium castaneum*	–	Stored grains	*In-vivo*	[93]
CuNPs	Spherical	103.94 nm	−2.2 mV	Photosynthesis with *Aloe vera* gel extract	*Tribolium castaneum*	–	Stored grains	*In-vivo*	[94]
ZnO-NPs	Spherical	20–70 nm	–	Controlled precipitation method	*Collectotricum* spp.	Anthracnose	*Coffea* spp.	*In-vitro*	[95]
SiNPs	Spherical	26.19 nm	–	Biosynthesis	*Aphis gossypii* Glover and *Phenacoccus solenopsis*	–	*Bt* cotton	*In-vivo*	[96]
Cu/Zn-NPs	Spherical	59.46–74.33 nm	51.1 mV	Photosynthesis with *Prosopis juliflora* leaf extract	*P. solenopsis*	–	Cotton	*In-vivo*	[97]
Chitosan NPs	–	–	–	–	*Puccinia triticina*	Wheat leaf rust	*Wheat*	Foliar	[91]
AgNPs and CuNPs	–	–	–	–	*Hemileia vastatrix*	Coffee leaf rust	*Coffea* spp.	Foliar	[92]
dA SeNPs and SiO_2NPs					*Alternatia alternata*	–	*Phaseolus vulgaris*	Foliar	[98]
ZnO-NPs	–	–	–	Photosynthesis with neem extract	*Puccinia triticina*	Wheat leaf rust	*Triicum aestivum*	Foliar	[99]
AgNPs	Rectangular to spherical	4–30 nm	–	Photosynthesis with *Moringa oleifera* leaf extract	*Puccinia striiformis*	Wheat stripe (yellow) rust	Wheat	Foliar	[100]
SeNPs	Spherical	61 nm	–	Photosynthesis with *Melia azedarach* leaf extract	*Puccinia striiformis*	Wheat stripe (yellow) rust	Wheat	Foliar	[101]

(Continued)

Table 1 (continued)

Nanoparticle	Morphology	Size particle	Zeta potential	Synthesis route	Phytopathogen	Disease	Phytopathogen host	Application	Ref.
CeONPs	Spherical	74.25 nm	–	Photosynthesis with *Acorus calamusas* rhizomes extract	*Puccinia striiformis*	Wheat stripe (yellow) rust	Wheat	Foliar	[101]
TiO_2 NPs	Round and clustered form (T1 and T3). Round, clustered, and porous form (T2)	15 nm	–	Sol-Gel (T1), T2, and T3 by photosynthesis with *Trianthema portulacastrum* (T2) and *Chenopodium quinoa* (T3) plant extracts	*Ustilago tritici*	Wheat rust	Wheat	*In-vitro*	[102]
PE@CS/ CMCS-NPs	Spherical	183.23 nm	–	Ionic crosslink	*Coleosporium plumeriae*	Plumeria rust	*Plumeria* spp.	Foliar	[34]
Zein NP-OEE	Spherical	<200 nm	12.51 ± 2.48 mV	Nanoprecipitation	*Stenocarpella macrospora*	Dry rot	*Zea mays*	*In-vitro*	[103]
MsEO/ CSNPs	Spherical	200 nm	−0.136 mV	Ionic-gelation	*Callosobruchus maculates* and *Sitophilus granaries*	–	Stored legumes and stored grains	*In-vivo*	[104]
Poly(ε-caprolactone) EO-NPs	–	275.33–402.13 nm	−6.76 to −11.8 mV	Emulsification/ evaporation technique	*H. vastatrix*	CLR	*Coffea* spp.	Foliar	[105]
Chitosan loaded Cu NPs	–	173.0 ± 8.4 nm	27.4 ± 1.6 mV	Ionotropic gelation method	*H. vastatrix*	CLR	*Coffea* spp.	Foliar application in foliar discs	[106]

Note: Nomenclature: CuNP: Copper nanoparticles, ZnO-NPs: Zinc oxide nanoparticles, SiNPs: Silica nanoparticles, Cu/Zn-NPs: Bimetallic copper/zinc nanoparticles, AgNPs: Silver nanoparticles, MnNPs: Manganese nanoparticles, SeNPs: Selenium nanoparticles, CeONPs: Cerium oxide nanoparticles, TiO_2 NPs: Titanium oxide nanoparticles, PE@CS/CMCS-NPs Penconazole (PE) loaded in chitosan (CS) carboxymethyl chitosan (CMCS) nanoparticles, Zein NP-OEE: Zein nanoparticles loaded with orange essential oil (NP-OEE), MsEO/CSNPs: *Mentha spicata* essential oil (MsEO) loaded chitosan nanoparticles, Poly(ε-caprolactone) essential oils (EO) loaded nanoparticles.

Table 2: Reported phytotoxic effect of nanoparticles

Nanoparticle	Morphology	Size particle	Zeta potential	Synthesis route	Tested NPs dose	Phytotoxic effect	Tested plant	Application	Ref.
CuO-NPs	–	51.81 nm	–	–	0.0 to 75 mg L^{-1}	Seed germination reduction at 75 mg L^{-1} in MS media without soaking the seeds	*Echinacea purpurea*	Supplemented seeds with CuO-NPs	[117]
PbS NPs	Cubic	15 ± 6 nm	–	Chemical route	5 to 50 mg L^{-1}	Seed germination reduction and inhibition of root biomass synthesis in the tested doses	*Zea mays* seeds	Seed imbibition and hydroponic culture	[114]
ZnO-NPs	–	9 and 40 nm	−2.44 ± 0.16 mV and 3.60 ± 0.32 mV	–	300 mg L^{-1}	9 nm ZnO NPs accumulation in stem and root cells vacuole	*Zea mays*	Hydroponic	[115]
AgNPs	–	<100 nm	–	–	0–1000 μg mL^{-1}	Significative reduction in Chla, Chlb, and carotenoid content	*Solanum Lycopersicon*	Medium enrichment with nanoparticles dose	[118]
ZnO-NPs	–	<50 nm	–	–	0–1000 μg mL^{-1}	Reduction in root length at 1000 μg mL^{-1}. Significative reduction in Chla, Chlb, and carotenoid content	*Solanum Lycopersicon*	Medium enrichment with nanoparticles dose	[118]

(Continued)

Table 2 (continued)

Nanoparticle	Morphology	Size particle	Zeta potential	Synthesis route	Tested NPs dose	Phytotoxic effect	Tested plant	Application	Ref.
CuNPs	–	<25 nm	–	–	0–1000 μg mL^{-1}	Reduction in seed germination at 1000 μg mL^{-1} and reduction in root length at 100 μg mL^{-1}. Significative reduction in Chla, Chlb, and carotenoid content	*Solanum Lycopersicon*	Medium enrichment with nanoparticles dose	[118]
CuO-NPs	–	<50 nm	–	–	0–1000 μg mL^{-1}	Reduction in seed germination at 1000 μg mL^{-1} and reduction in root length at 100 μg mL^{-1}. Significative reduction in Chla, Chlb, and carotenoid content	*Solanum Lycopersicon*	Medium enrichment with nanoparticles dose	[118]
SNPs	Spherical	5–10 nm	–	Photosynthesis with aqueous extract of *Medicago polymorpha*	20 mg L^{-1}	Significative reduction in seed germination, vigor index, root and shoot length, fresh weight, chlorophyll, and carotenoid content	*Vigna radiata* L.	Seeds imbibition for 24 h and	[119]

Note: Nomenclature: CuO-NPs: Copper oxide nanoparticles, PbS NPs: Lead Sulfide nanoparticles, ZnO-NPs: Zinc oxide nanoparticles, AgNPs: Silver nanoparticles, CuNPs: Copper nanoparticles, SNPs: Silver nanoparticles, MS: Murashige and sooge free hormone media, Chla: Chlorophyll a, Chla: Chlorophyll b.

The possible toxicological effects of nanoparticles concern the application of nanoparticles in organic and sustainable crops. Organic agriculture takes care of the soil, ecosystem, and people's health [120]. Some requirements in organic and sustainable crop systems involve the integration of biological processes into the production chain, avoiding the use of toxic pesticides, reducing the use of non-renewable resources as much as possible, and implementing solutions derived from the farmer's experiences and skills [120,121]. Since organic agriculture involves the use of innovative and scientific measurements combined with traditional practices, several studies must be done before applying nanoparticles to coffee farms with organic and sustainable systems [120–123].

To comply with organic and sustainable crop practices, it is important to assess the security of the crop environment. Before starting with applications of nanoparticles a comprehensive literature revision must be done. Understanding the action mechanism and the physicochemical properties of nanoparticles improves the comprehension of nanoparticle interactions. These interactions are related to how the nanoparticles impact the interest in phytopathogen, host plant, soil, and soil microbiome. The application of nanoparticles in phytopathogen control can be accompanied by biological control and sustainable crop practices with a complete overview of the nanoparticle's impact on the farm environment.

The application of nanoparticles in the control of *H. vastatrix* germination is not been explored at now but has extreme potential in research, e.g., the phytotoxicity and safety amounts and types of nanoparticles in different phenological states of coffee plants or coffee seeds germination and how the nanoparticles applications can induce different responses in photosystems, secondary metabolites synthesis, and enzymatic activity or how nanoparticles can induce defense response pathways in coffee plants.

7 Conclusion

The disease caused by *H. vastatrix* represents a great problem phytosanitary in coffee plants that affects sustainable coffee production all over the world. Although there are various strategies for its control, it is a fact that integrated management is required to control this disease. In this sense, the use of new tools such as nanotechnology could be a possible alternative in the control of the disease. Therefore, future studies need to address an integrated approach to managing the disease with an international exchange of knowledge and biological materials and the implementation of new technologies.

Acknowledgement: The author acknowledges the Consejo Nacional de Humanidades, Ciencias y Tecnologías CONHACyT for the support offered to his studies as a postgraduate student from Doctorado en Ciencias Agrícolas at the Instituto de Ciencias Agrícolas in Universidad Autónoma de Baja California.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: The study was conceived and designed by A. Salazar-Navarro, V. Ruiz-Valdiviezo, J. Joya-Davila, and D. Gonzalez-Mendoza. The manuscript was drafted by A. Salazar-Navarro. Funding was obtained by V. Ruiz-Valdiviezo, J. Joya-Davila, and D. Gonzalez-Mendoza. The study was supervised by D. Gonzalez-Mendoza, V. Ruiz-Valdiviezo, and J. Joya-Davila. All authors have reviewed and approved the published version of the manuscript.

Availability of Data and Materials: The data utilized in this study have been incorporated within the tables and pictures.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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CAPÍTULO 4. Síntesis de nanopartículas de sílice funcionalizadas con oligómeros de quitosano

Título original del artículo: Synthesis of Silica Chitosan Oligosaccharides Nanoparticles

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Este capítulo corresponde a un artículo publicado en: Agro Productividad, Vol. 16(11), 2023, pp. 107–114. https://doi.org/10.32854/agrop.v16i11.2728 *(El contenido se presenta en su idioma original con fines académicos).*

Resumen

En este estudio se sintetizaron nanopartículas de sílice funcionalizadas con oligómeros de quitosano (SiNPs-COS) mediante hidrólisis química y mecánica asistida por microondas del quitosano, seguida de interacción electrostática con SiNPs obtenidas por el método Sol-Gel. Se obtuvieron dos formulaciones (A y C) con tamaños de partícula de 251.8 nm y 139.35 nm, respectivamente, y potencial zeta superior a +30 mV, lo que indica buena estabilidad coloidal. Las nanopartículas mostraron actividad antimicrobiana contra *Escherichia coli* y *Staphylococcus aureus*, destacando la formulación C por su mayor eficacia. Se concluye que los sistemas COS-SiNPs presentan propiedades fisicoquímicas adecuadas y potencial como plataforma para aplicaciones agrícolas.

Palabras clave: quitosano, sílice, Sol-Gel, hidrólisis química, hidrólisis mecánica.

Abstract

Objective: To obtain chitosan oligosaccharides (COS) and evaluate COS uses for the obtention of nanosystem based on silica as vehicle and compare the COS-silica nanosystem with the chitosan (Chi) precursor system as Chitosan-silica nanosystems.

Design/methodology/approach: A combination of hydrolysis chemical and mechanical (microwave assisted) were used to obtain COS with the oxidative action of hydrogen peroxide. Sol-Gel adapted method was used to synthetize silica nanoparticles (SiNPs) from sodium metasilicate and the electrostatic interactions between SiNPs and Chi/COS were used to functionalize the SiNPs surface with Chi/COS.

Results: Nanosystem composed from COS and SiNPs were obtained successful as A COS-SiNPs and C COSSiNPs with particle size of 139.35 nm and 251.8 nm and zeta potential of 30.40 mV and 34.67 mV respectively with antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.*

Limitations on study/implications: Stabilize the systems compound of chitosan-silica nanoparticles due to the molecular weight of chitosan which loss the stabilized the SiNPs suspension and due the incompatibility of both systems pH.

Findings/conclusions: COS and COS-SiNPs stable systems were obtained with an improvement of the antimicrobial activity of the system in contrast of Chi-SiNPs systems.

Keywords: chitosan, silicon, Sol-Gel. Chemical hydrolysis, mechanical hydrolysis, microwave assisted.

Colegio de Postgraduados

Synthesis of silica chitosan oligosaccharides nanoparticles

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ABSTRACT

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Citation: Salazar-Navarro, A. A., Rivera-Reyna, N. E., & González Mendoza, D. (2023). Synthesis of silica chitosan oligosaccharides nanoparticles *Agro Productividad*. https://doi.org/10.32854/agrop.v16i11.2728

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: June 19, 2023.
Accepted: October 05, 2023.
Published on-line: December 27, 2023.

Agro Productividad, 16(11). November. 2023. pp: 107-114.

INTRODUCTION

Chitosan is considered as the second most abundant polysaccharide followed by cellulose; chitosan is a biodegradable biopolymer derived from chitin by a deacetylation process. Chitin/Chitosan can be obtained from crustaceous exoskeleton, insects, algae, and from the cell wall of some fungus. Chitin is deacetylated and depolymerized to obtain chitosan in alkaline conditions where acetyl groups are delinked from the saccharide chain, thus reducing the affinity of the polysaccharide to stay attached making chitosan more water soluble and less viscous [1]. Chitin and chitosan are compounded from the same saccharide monomers whit the main difference in the proportion of the units in the polysaccharide structure, chitin is mostly compound with N-acetyl-D-glucosamine (GlcNAc) with a portion of D-glucosamine units (GlcN) and the saccharide units are joined by a β 1-4 glycosidic bond. In contrast, chitosan is mainly compound by GlcN units with a about 10% content of GlcNAc, the ration between GlcN and GlcNAc units defines the deacetylated grade of chitosan [2-4].

Furthermore, chitosan biodegradability other properties have been studied the last decades, as chitosan biocompatibility, low toxicity, antimicrobial effect due their amine groups ($-NH_2$), also chitosan have been studied in agricultural fields due their activity as elicitor in plant defense mechanisms hence chitosan can act as antimicrobial biopolymer coating which activates some defense mechanisms in plants as ethylene and salicylic acid pathways, two of the most important signaling events involving in the resistance of some plant-pathogen interactions as coffea species with *Hemileia vastatrix* (biotrophy pathogen cause coffee leaf rust disease) [5, 6].

The main properties who define the behave and applicability of chitosan are the deacetyl grade, the molecular weight, the availability of amine groups (NH_2) and the zeta potential due the electrostatic charge difference between the biopolymer and the pathogens cell wall [2, 3]. The chitosan can be categorized in function of their molecular weight (Mw) as low molecular weight when Mw<100 kDa, medium molecular weight when Mw<100-1000 kDa and high molecular weight when >1,000 kDa [4].

Besides the widely chitosan applications have inconvenient to scale and improve applications in nanosystems, chitosan solution usually shows high viscosity values and low water solubility in absent of acid environments which reduce their viability to design nanosystems with chitosan, especially if the vehicle particle has more stability in alkaline environments as silica nanoparticles obtained from sodium metasilicate [4, 7].

In contrast chitosan oligosaccharides (COS) have low viscosity values related to their low molecular weight lower than 3.9 kDa and their low polymerization grade, as the COS are obtain from Chitosan by the glycosidic bond hydrolysis, the COS conserve the saccharide units GlcN and GlcNAc which conform chitosan chain but in less length chain and with more functional groups available who are related to COS more water solubility in neutral pH solution with less viscosity, the hydrolysis of glycosidic bond results saccharide fragments of the original chain, at the breaking sites one fragment results in hydroxyl group and the other in aldehyde group [4, 8].

COS can be obtained by chemical, mechanical or enzymatic hydrolysis. Chemical hydrolysis can be divided into acid and oxidative hydrolysis. In acid hydrolysis high concentrations of acids is used where the break of glycosidic bond starts in the protonation of oxygen in glycosidic bonds followed by the group reduction by the addition of water molecule which results in the decomposition of the glycosidic [4, 9, 10]. The oxidative hydrolysis is bases in the uses of hydrogen peroxide to produced hydroxyl radicals due H_2O_2 high instability and attack the glycosidic linkages of chitosan [4, 11].

Mechanical hydrolysis can be combined with acid or oxidative hydrolyses to accelerate the reaction. Mechanical methods can be found microwave and gamma radiation assisted methods where the microwave assisted hydrolysis is less efficient in contrast to gamma radiation but significantly reduces their cost operation, environment impact and scalability. Microwave assisted operates in two ways can generate shear stress and thermal degradation of the polysaccharide [4, 9, 12].

Nanomaterials has gained research attention recently due their wide application in several industries due their behavior and activity change at nanoscale in contrast of their bulk material properties and due it versatility to design more nanomaterials to obtain different morphologies, arrangements or surface modification [13-15], especially in agronomy has ben used to control plant pathogens as insects *e.g.* to control *Tribolium castaneum* with copper nanoparticles (CuNPs) [16-18], and other diseases as Coffee Leaf Disease (CLF) caused by *Hemilea vastatrix* [5], additionally, nanomaterials has been used un food packaging to improve food quality [19], and to improve seed germination [20-22].

SiNPs has special attention due its versatility to obtained in different shape, arrangement and surface chemistry, SiNPs can be obtain from several methods and silicon sources, the mainly used methods are based on Sol-Gel synthesis as the main bottom-up method used due its facilities to control the surface particle chemistry, shape, surface charge and particle size and usually silicon metasilicate or tetraethyl orthosilicate are used as silicon source [7, 13, 19, 23, 24].

MATERIALS AND METHODS.

Silica nanoparticles synthesis

Silica nanoparticles (SiNPs) were synthetized as we report previously by Sol-Gel method [7] sodium metasilicate 0.3M solution distilled water (Metso pentabead 20®, commercial grade) was used as silicon source, the sodium metasilicate solution was filtered with ion-exchange resin to decrease the sodium ions in the solution and reduce the pH from 14 to 10, followed 5 ml of ethanol (Fermont®, analytic grade) was added to catalyzed the condensation of siloxane bonds and leave 15 min in low stirring. The previous solution was homogenized in 2,000 rpm for 30 min with PEGlynated silicon surfactant with methoxy terminal groups (Silwet® L-77, Momentive) to improve micelle formation, finally the obtained suspension was treated in 105 °C reflux for 30 min to improve stability to the suspension.

Silica-chitosan nanoparticles synthesis

Low molecular weight chitosan (Sigma-aldrich®) was dissolved at 3 wt% in previously prepared acetic acid (Fermont®, analytic grade) 1% in distilled water at 70° in vigorous stirring until the chitosan was completely dissolved. To synthetize the silica-chitosan nanoparticles (SiChiNPs) the previously chitosan solution was added into the homogenization step described above with the treated sodium metasilicate and PEGlynated silicon surfactant.

Silica-chitosan oligosaccharides nanoparticles synthesis

To synthetize silica-chitosan oligosaccharides nanoparticles (SiCOSNPs) the previously synthetized SiNPs was dispersed in distilled water at 0.5% with slow stirring, followed obtained COS was added in two different final concentrations 0.5% and 1% and let in agitation for 1 h, the obtained suspensions were labeled as ASiCOSNPs and CSiCOSNPs respectively.

Characterization

The particle size and zeta potential were obtained in particle size analyzer Litesizer 500 (Anton Paar®). The IR spectrum was obtained in ATR-FTIR 4300 (Agilent®), the samples were dried above the ATR to avoid the water molecules interference. The viscosity was obtained at 25 °C by viscosimeter VISCO QC 100 (Anton Paar®). The inhibitory effect of obtained nanoparticles was analyzed by antibiogram method in disposable Petri dishes with previously prepared Mueller-Hinton agar (BD Bioxon®) against *Escherichia coli* ATCC® 25922 and *Staphylococcus aureus* ATCC® 25923.

RESULTS AND DISCUSSION

Viscosity

The viscosity measurements (Table 1) show a significative decrement from the original chitosan solution when the hydrogen peroxide (Table 1. COS H_2O_2) was added from 41.60 P to 3.2 P and more decrement after the microwave treatment (Table 1. COS H_2O_{2-m}) to 32.39 cP. The viscosity decrement can be related to the reduction of molecular weight of the chitosan and the results shows after the microwave treatment confirm the synergic degradation by the combination of chemical and mechanical hydrolysis to obtain chitosan oligosaccharides [8, 10-12].

Fourier-transform infrared spectroscopy (FTIR)

The IR spectrum show similar behave between the dried chitosan spectrum (chi s) and the dried chitosan oligosaccharide spectrum (cos s) with the main difference of the cos s spectrum shows more definition in it peaks spectrum, which according with [25] this can be related to the increase of available functional groups due the glycosidic bond break. Both spectrums start at 650 cm^{-1} regions with stretching of C-O-C and C-O-H bonds at 656.7 cm^{-1} and 652.2 cm^{-1} for chi s and cos s respectively which can be related to the breaking of glycosidic bonds and protonation of the CO^- groups [25].

Both spectrums show well define peaks at 887.1 cm^{-1} (chi s) and 890.8 cm^{-1} (cos s) with similar transmittance which with the peaks at 1012-1016 cm^{-1}, 1052-1060 cm^{-1}, and 1152 cm^{-1} are related with the habitual behave of saccharide structure [26, 27]. The peaks at 1258-1262 cm^{-1} and 1374-1377 cm^{-1} can be related with the presence of amide III associated with GlcNAc units [1, 26-29], the peaks at 1403-1420 cm^{-1} are related to bend vibration of O-H and C-H bonds [1, 26, 27, 30], the presence of peaks around 1531-1539 cm^{-1} are related to vibrations of amide II and C-OH bonds [1, 26, 27], the peaks in 1632-1634 shows the presence of amide I and the peaks around 1700-1702 cm^{-1} are related with carboxyl group vibrations and the peaks at 2866-3069 are related to O-H and C-H bond vibrations [1, 26, 27].

Table 1. Dynamic viscosity related to COS obtention from chitosan.

Sample	Viscosity (Pa)
Chitosan	41.6
COS H_2O_2	3.2
COS H_2O_{2-m}	0.3239

COS H_2O_2 are for COS obtained by oxidative hydrolysis with hydrogen peroxide and COS H_2O_{2-m} for COS obtained by oxidative and mechanical hydrolysis.

The nanoparticles systems synthetized with silica nanoparticles do not show significant differences with the previously discussed spectrums which can be due to a signal overlap between similar bond energy. The siloxane bonds (Si-O-Si) could be overlapped by the glycosidic bond in the saccharide (C-O-C) at 467-586 cm^{-1} respectively and in 1000-1062 cm^{-1} peaks, the peak at 892 cm^{-1} also can be attributed to the bonds Si-C and NH_2, the peaks at 2000-2332 cm^{-1} can be related to S-H or C=O bonds [7, 25, 31].

Dynamic Light Scattering Analysis

Dynamic Light Scattering Analysis shows an increment in size of nanoparticles silica (Figure 2. A) well defined and monodispersed histogram presents a particle size of 6.74 nm with zeta potential (ζ) of −32.80 mV related to deprotonated hydroxyl groups rich in the surface of SiNPs. The first A-Si-COSNPs (Figure 2. B) shows an increment in particle size to 251.8 nm with ζ=30.40 mV which reflects the formation of coat made of COS at the surface of SiNPs. Similar results are shown by C-Si-COSNPs (Figure 2. C) with particle size increment to 139.35 nm and ζ=34.67 mV. By DLS results the nanosystem synthetized with 0.5% of COS (C-Si-COSNPs) can be correlated to more effective and stable nanosystem thus related to the increment of potential zeta in 4.27 mV which reflects more repulsive forces between particles and the smaller particle size (139.35 nm) improve the efficiency of the nanosystem. Additionally, the nanosystem C-Si-COSNPs shows a distribution of size particles more related to monomodal nanoparticles system. The DLS results in Chi-SiNPs systems were discarded due to the trend of the system to precipitate due to molecular weight and incompatible pH regions of chitosan an SiNPs [7].

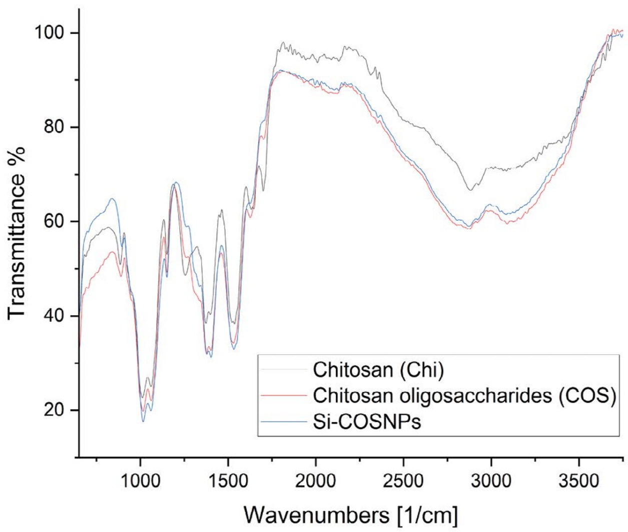

Figure 1. ATR-IR spectrums for Chitosan (Chi), Chitosan oligosaccharides (COS) and Silica-Chitosan Oligosaccharides Nanoparticles (Si-COSNPs).

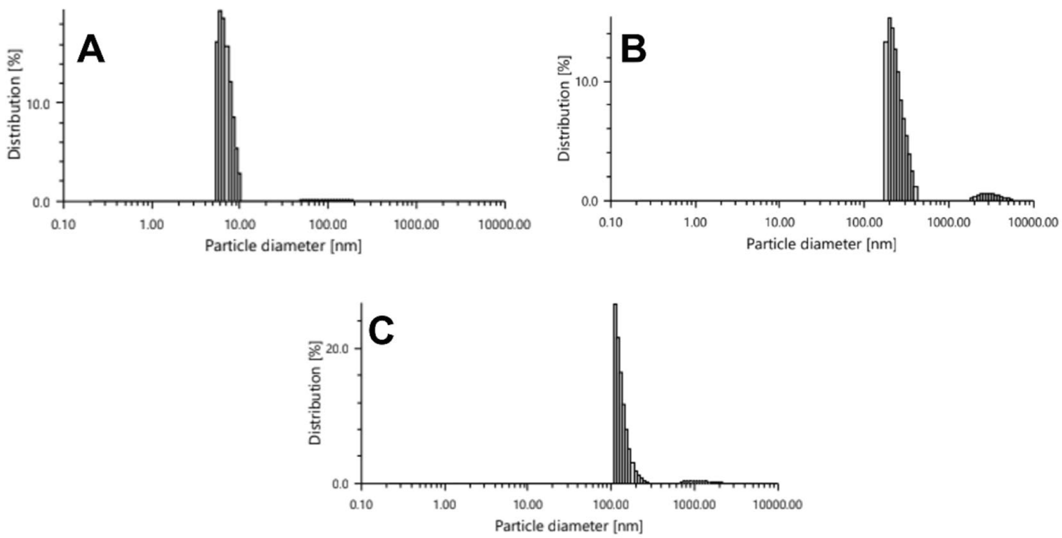

Figure 2. Particle size histogram for SiNPs (A), A-Si-COSNPs (B) and C-Si-COSNPs (C).

Inhibitory efficacy - antibiograms

The obtained chitosan oligosaccharides show inhibitory diameter significant bigger at the 24 and 48 h after the inoculation with *E. coli* and *S. aureus* then the chitosan solution and then silica nanoparticles (Table 2), the silica chitosan oligosaccharide nanosystems (A and C) also show significant inhibitory efficacy in contrast of the silica chitosan nanosystem. The improvement of antimicrobial activity of COS and COS-SiNPS can be related to the increment of amine and amide groups available after hydrolysis of glycosidic bonds, especially due to the increase of primary amino groups [32]. Also, pristine silica nanoparticles show zero-antimicrobial activity as was expected due SiNPs surface negative charge, in contrast, due the positive surface charge shows by COS and COS-SiNPs can be attracted and bonded to bacterial cell wall with negative charge due the carboxylic acid groups and this interaction can makes an impermeable coat of COS or COS-SiNPs blocking the nutrient uptakes and leading to the cell death [32, 33].

Table 2. Antibiogram results of tried nanosystems and precursors against *Escherichia coli* and *Staphylococcus aureus*.

Sample	***Escherichia coli***		***Staphylococcus aureus***	
	24 h (mm)	**48 h (mm)**	**24 h (mm)**	**48 h (mm)**
SiNPs	0	0	0	0
Chitosan	1.9	1.4	1.9	1.4
COS	2.8	2.6	2.8	2.6
SiNPs-COS A	1.9	1.8	1.9	1.8
SiNPs-COS C	1.7	1.6	1.7	1.6
SiNPs-Chi	0	0	0	0

CONCLUSIONS

A stable silica chitosan oligosaccharide nanosystem was successfully obtained with antimicrobial activity against *E. coli* and *S. aureus* by the synthesis of COS from low molecular weight chitosan by chemical and mechanical hydrolysis with hydrogen peroxide and 700W microwave. The obtained nanosystems maintain their main chemical composition characteristic of chitosan with more availability of functional groups due the breaking of glycosidic bond during the COS synthesis. The obtained COS-SiNPs shown a particle size 139.35 nm and ζ=34.67 mV with growth inhibitory diameter of 1.9 mm and 1.8 mm against *E. coli* 24 h and 48 h the inoculation, and 1.9 mm and 1.8 mm against *S. aureus* 24 h and 48 h the inoculation.

ACKNOWLEDGEMENTS

The author acknowledges to the Consejo Nacional de Humanidades, Ciencias y Tecnologías CONHACyT for the support offered to his studies as a postgraduate student from Doctorado en Ciencias Agicolas at Instituto de Ciencias Agricolas in Universidad Autónoma de Baja California.

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CAPÍTULO 5. Respuesta bioquímica de *Coffea arabica* var. *Bourbon* a la aplicación foliar de oligómeros de quitosano

Título original del artículo: *Coffea arabica* var. Borbon Biochemical Response to Chitosan Oligosaccharides Foliar Exposure

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Artículo publicado en: **Phyton, International Journal of Experimental Botany**, Vol. 94(3), 2025, pp. 401–412. https://www.techscience.com/phyton/v94n3/60217 *(El contenido se presenta en su idioma original con fines académicos).*

Resumen

Se evaluó la respuesta bioquímica de *Coffea arabica* var. *Bourbon* a la aplicación foliar de quitosano y oligómeros de quitosano (COS) sintetizados por hidrólisis química y física. Los tratamientos incluyeron distintas concentraciones de COS y quitosano, y se midieron proteínas solubles, fluorescencia de clorofila, y la actividad de enzimas asociadas a defensa (PAL, catalasa, quitinasa, β-1,3-glucanasa y peroxidasa). Las aplicaciones de COS mostraron mayor actividad enzimática que el quitosano, especialmente en concentraciones del 0.25% y 0.5%. Además, se observó una mayor eficiencia fotosintética transitoria en las primeras 24 h. Los resultados sugieren que el COS tiene un efecto elicitor más eficiente, sin efectos fitotóxicos visibles, y podría usarse como estrategia preventiva contra estrés biótico en café.

Palabras clave: actividad enzimática; PAL; β-1,3-glucanasa; quitinasa; fluorescencia de clorofila; fotosistema II

Abstract

The biochemical response of *Coffea arabica* var. *Bourbon* to chitosan and chitosan oligosaccharides (COS) was evaluated in one-year-old plants under greenhouse conditions. COS solutions were synthesized through chemical and physical hydrolysis using acetic acid, hydrogen peroxide, and microwave irradiation. The obtained COS had an average molecular weight (Mw) of 3549.90 $\pm$ 0.33 Daltons (Da), a deacetylation degree (DD) of 76.64 $\pm$ 1.12%, and a polymerization degree (PD) of 18.91 $\pm$ 0.0018. Solutions of chitosan and COS were applied to C. arabica var. *Bourbon* at concentrations of 0.25, 0.5, and 1 wt%. The experimental design was conducted using a completely randomized design with four replications. The biochemical responses assessed included soluble protein content, phenylalanine ammonia-lyase (PAL), chitinase, β-1,3-glucanase, peroxidase, catalase, and chlorophyll fluorescence. The application of COS demonstrated significant differences ($\alpha = 0.05$) in protein concentration, with the activity of β-1,3-glucanase, chitinase, and catalase being 1.5, 7.5, and 3.9 times higher, respectively, while showing similar behavior to chitosan in PAL activity, both up to 4.4 times higher than the distilled water control and lower than chitosan in peroxidase activity. Treatments with chitosan yielded a higher photochemical efficiency of Photosystem II (PSII). The application of COS suggests a viable foliar alternative to active plant defense mechanisms without the risk of phytotoxicity.

Keywords: Enzymatic activity; PAL; β-1,3-glucanase; chitinase; chlorophyll fluorescence; photosystem II

Phyton-
International Journal of
Experimental Botany

Tech Science Press

Doi:10.32604/phyton.2025.062871 Published Online: 19 March 2025

ARTICLE

Coffea arabica var. Borbon Biochemical Response to Chitosan Oligosaccharides Foliar Exposure

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Received: 30 December 2024; Accepted: 20 February 2025

ABSTRACT: The biochemical response of *Coffea arabica* var. Borbon to chitosan and chitosan oligosaccharides (COS) was evaluated in one-year-old plants under greenhouse conditions. COS solutions were synthesized through chemical and physical hydrolysis using acetic acid, hydrogen peroxide, and microwave irradiation. The obtained COS had an average molecular weight (Mw) of 3549.90 ± 0.33 Daltons (Da), a deacetylation degree (DD) of 76.64 ± 1.12%, and a polymerization degree (PD) of 18.91 ± 0.0018. Solutions of chitosan and COS were applied to *C. arabica* var. Borbon at concentrations of 0.25, 0.5, and 1 wt%. The experimental design was conducted using a completely randomized design with four replications. The biochemical responses assessed included soluble protein content, phenylalanine ammonia-lyase (PAL), chitinase, β-1,3-glucanase, peroxidase, catalase, and chlorophyll fluorescence. The application of COS demonstrated significant differences ($\alpha = 0.05$) in protein concentration, with the activity of β-1,3-glucanase, chitinase, and catalase being 1.5, 7.5, and 3.9 times higher, respectively, while showing similar behavior to chitosan in PAL activity, both up to 4.4 times higher than the distilled water control and lower than chitosan in peroxidase activity. Treatments with chitosan yielded a higher photochemical efficiency of Photosystem II (PSII). The application of COS suggests a viable foliar alternative to active plant defense mechanisms without the risk of phytotoxicity.

KEYWORDS: Enzymatic activity; PAL; β-1,3-glucanase; chitinase; chlorophyll fluorescence; photosystem II

1 Introduction

The global coffee trade is one of the most important commodity markets worldwide [1]. In 2023, coffee production reached 171.4 million bags (60 kg), with Brazil as the leading coffee-producing country, followed by Vietnam. Mexico ranked 10th in global coffee production [2], with 81%–82% of its output concentrated in the states of Chiapas, Veracruz, and Puebla [3]. In 2023, approximately 87% of Mexico's total coffee production was *Coffea arabica*, while the remaining percentage was *Coffea canephora*, commonly known as arabica and robusta, respectively [2]. *C. arabica* generates higher income in Mexico due to its cup qualities, but it also presents production challenges due to its susceptibility to pests and diseases [4]. For instance, Coffee Leaf Rust (CLR), caused by *Hemileia vastatrix*, an obligate biotrophic fungus, and Coffee Berry Disease (CBD), caused by *Colletotrichum kahawae* Waller & Bridge [5], are among the most threatening diseases for coffee plantations. *H. vastatrix* infections in *C. arabica* plantations can cause yield losses of up to

70% for farmers. In 2016, Mexico reported a 50% loss in *C. arabica* production due to the severity of the CLR epidemic [6]. Several coffee varieties grown in Mexico, such as Typica, Bourbon, Caturra, Mundo Novo, and Garnica, are known for their susceptibility to CLR [7].

Some alternatives for controlling phytopathogens in coffee plantations involve cultural practices as well as chemical and biological control methods. Cultural practices involve modifying plantation management to create conditions that limit the spread of phytopathogens [6,8]. These measures include reducing plantation density and implementing a canopy crop program to minimize microenvironments and direct contact between plants [9], maintaining a younger population of coffee plants, and ensuring proper plant nutrition with acidified soil to enhance nutrient uptake [8]. Additionally, coffee plantation management could also include establishing physical barriers to protect the plant population from wind, adverse temperature conditions, and the spread of pathogen inoculum [8,10]. Natural barriers also facilitate the implementation of these physical barriers and biological control strategies by enriching of soil microbiota [8,10,11].

Chemical control involves the application of exogenous substances to coffee plants, either soil treatment or foliar spraying. Some pesticides used in coffee plantations belong to chemical families such as carbamates, neonicotinoids, triazoles, and organophosphates [12]. These substances can act as protective or systemic fungicides [13]. Exogenous elicitors are other substances used to control phytopathogens, which do not necessarily possess direct fungicidal activity [14]. Elicitors are molecules recognized by patter recognition receptors (PRRs) that activate the plant's defense mechanisms. Elicitors can be sourced from microbial secretions or from the wound tissues of host plants. Some alternatives for controlling phytopathogens include foliar applications of exogenous elicitors related to the target pathogen as a protective strategy [6].

The primary mechanism in biological and chemical control by elicitors involves activating the plant host defense mechanisms. Plants react to microorganism invasion with biochemical changes associated with biotic stress signaling, which alters their physiology. For instance, available oxygen can be utilized to produce Reactive Oxygen Species (ROS) that inhibit pathogen growth [5,15]. Additional defense strategies include cell wall thickening, oxidative bursts, hypersensitive responses (HR), the synthesis of antimicrobial compounds like phenolics, flavonoids, and lignins, and the expression of antifungal pathogenesis-related proteins (PR) such as chitinases and glucanases, as part of their immune response [5,15–17].

Chitosan is derived from chitin under alkaline conditions through a process known as deacetylation. While both chitosan and chitin are made up of the same monomer units, they differ in their proportions. They consist of N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) linked by a β-1,4-glycosidic bond. Chitin is primarily composed of GlcNAc units, whereas chitosan mainly comprises GlcN units [18,19]. The synthesis of chitosan oligosaccharides (COS) involves breaking glycosidic bonds and depolymerizing chitosan through methods such as acid hydrolysis, oxidative hydrolysis, mechanical hydrolysis, or enzymatic hydrolysis [19]. COS is defined as chitosan with a degree of polymerization (DP) of less than 20 and a molecular weight (Mw) of under 3900 [20]. COS has gained popularity in agricultural applications due to its enhanced water solubility, low viscosity, and stability at neutral pH [19,21].

Chitosan is recognized by PRRs and induces the transcription of defense-related genes, such as β-1,3-glucanases, chitinases, PAL, PR1, and antioxidant enzymes [22]. It also activates the SA pathway and SAR response through the activation of gene NPR1 transcription, a key gene that regulates plant defense mechanisms across wide variety of plants [22].

The molecular and enzymatic effects of chitosan and chitosan derivatives have been studied in various *C. arabica* varieties to mitigate biotic and abiotic stressors [23]. Previous studies have identified key physicochemical parameters that influence the molecular and biochemical responses of *C. arabica* as the source of chitosan, production method, viscosity, molecular weight, and degree of polymerization [22]. In contrast, fewer studies have been conducted to describe the molecular and biochemical impact of COS on *C. arabica* varieties. This highlights the need for a properly characterization of COS and understanding of how plants respond to its application.

Various biochemical assays can be achieved to properly understand how the exposed plants responded to an exogenous elicitor such as chitosan and derivatives. Beside them can be found gene expression, enzymatic activity, photopigments concentration, and chlorophyll (Chl) fluorescence. Chl fluorescence is widely used in plant studies to examine the effects of biotic and abiotic factors on the plants [24–26]. The relationship between variable and maximum fluorescence is referred to as maximum PSII efficiency and is employed to assess the photosynthetic effectiveness of PSII. The expected range for Fv/Fm is about 0.80 to 0.83. In coffee plants, the optimal value is 0.85; however, this value may decrease to 0.79 under stress conditions [21,27].

Chl fluorescence serves as a sensitive tool used to assess and monitor the physiological and biochemical state of plants. It is employed to observe how external factors (e.g., stressors, application of chemicals, changes in the rhizosphere microbiome) affect the stability and efficacy of PSII [25,26]. This technique can help elucidate and understand potential fluctuations in PSII due to inhibition in the electron transfer chain or physical damage in the chloroplast [25,26].

Previous studies have demostrated that the foliar application of chitosan increases the expression of plant defense genes and enzymatic activity *in C. arabica* [17,22]. The application of COS foliar activated plant defense mechanisms in the *Arabidopsis* ecotype Columbia [28], in *Nicotiana glutinosa* using a Cytosin-peptidemycin and COS system (CytPM-COS) [29], and in *Passiflora* spp. [30]. However, the effects of foliar application of chitosan oligosaccharides have not been fully studied in *C. arabica* varieties, except for the effectiveness of COS against *H. vastatrix*, evaluated in foliar discs [31]. The objective of this study was to evaluate the biochemical responses of *C. arabica* var. Borbon to the foliar application of chitosan oligosaccharide (COS). This research aims to enhance the understanding of COS applications and its potential to strengthen the resistance of *Coffea* spp. against plant pathogens.

2 Materials and Methods

2.1 Synthesis of Chitosan Oligosaccharides (COS)

COS was synthesized according to [19] from low molecular weight chitosan with a deacetylation level greater than 75%, which was dissolved in 1% acetic acid at a concentration of 3 wt% while stirring at 500 rpm and 70°C until the chitosan was fully dissolved. Oxidative and mechanical hydrolysis were performed to break the β-1,4-glycosidic bond by adding 1% hydrogen peroxide dropwise at 25°C while stirring at 300 rpm, followed by microwave application at 700 W for 1.5 min [21].

2.2 Chitosan Oligosaccharides Characterization

2.2.1 Deacetylation Degree (DD)

Deacetylation Degree determination was performed by Fourier Transform Infrared Spectroscopy (FTIR) according to [17,32,33] by FTIR with Attenuated Total Reflectance (ATR) (Agilent 4300 Handheld FTIR, USA) from 4000 to 650 cm^{-1} with 2 cm^{-1} resolution, 10 µL of each sample was dried over ATR. The *DD* was obtained by Eqs. (1) and (2), where A_{1320} and A_{1420} are the absorbance of chitosan at wavelengths 1320 and 1420 cm^{-1}, respectively, and *DA* is the Degree of Acetylation [17].

$$DA\,(\%) = \frac{0.3822 - \frac{A_{1320}}{A_{1420}}}{0.03133} \tag{1}$$

$$DD\,(\%) = 100\% - DA(\%) \tag{2}$$

2.2.2 Molecular Weight Determination

Molecular weight determination was carried out using intrinsic viscosity, as described in [34]. The viscosities of chitosan and COS were measured with an Ostwald capillary viscometer at 25°C, using a solvent system of 0.3 M acetic acid and 0.2 M sodium acetate. The molecular weight was calculated utilizing the Mark-Houwink Eq. (3), where $[\eta]$ represents the intrinsic viscosity, M_V denotes the viscosity average molecular weight, and K and a are constants specific to a given solvent system. The Degree of Polymerization (PD) was determined from the average molecular weight and the proportional contributions of the molecular weights of chitosan monomers N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN).

$$[\eta] = KM_v^a \tag{3}$$

2.3 Plant Materials and Experimental Design

A local producer in Angel Albino Corzo, Chiapas, Mexico, provided *Coffea arabica* var Borbon seeds. The seeds were previously disinfected with 20% commercial sodium hypochlorite for 5 min and rinsed with 20 mL of sterile distilled water three times to eliminate traces of sodium hypochlorite. They were then dried at room temperature, sown in sterile sand, and transplanted two months before the analysis to ensure the acclimatization of the plants. Six-month-old transplanted plants were grown in polyethylene bags measuring 6.7 cm × 6 cm × 26 cm (Length × Width × Height) with a substrate mixture of peat moss and agrolite in a 3:1 ratio. Under greenhouse conditions, the plants were watered twice a week with 100 mL of water. Sixty-eight uniform plants were selected to perform the COS foliar application. The selected plants were randomly exposed to different treatments, including a distilled water control and different concentrations of chitosan and synthesized COS (1%, 0.75%, 0.5%, and 0.25%) through foliar application of 2 mL 24 h before each analysis. All treatments were evaluated with four replicates.

2.4 Enzymatic Activity

The second pair of leaves from each plant was collected to perform protein extraction as outlined in [35]. The resulting protein extract was used to assess the soluble protein content (SPC) using the Bradford method. The SPC values obtained were utilized to determine enzyme-specific activity. The enzymatic activities assessed included PAL, β-1,3-glucanases, chitinases, peroxidase (POD), and catalase (CAT). SPC and enzymatic activities were analyzed using UV-Vis spectroscopy (Nanodrop One®, Thermo Fisher Scientific, Waltham, MA, USA).

2.4.1 Enzymatic Extraction

Protein extraction was performed according to [35] by homogenizing 50 mg of collected leaves in liquid nitrogen using pre-chilled mortar and pestles and macerating for 4 h at 4°C in an extraction buffer composed of Tris-HCl 0.1 pH 8, ascorbic acid 0.1 wt%, glycerin 10%, polyvinylpyrrolidone (PVP) 1 wt%, and β-mercaptoethanol 5%. The homogenized samples were centrifuged at 15,000× g for 15 min. The supernatant from each sample was then transferred to microcentrifuge tubes and stored at −4°C until further use.

2.4.2 Phenylalanine Ammonia Lyase (PAL) Activity

PAL activity was determined as detailed in [36], with a few modifications. 5 μL of enzyme extract were added to 145 μL of Tris-HCl buffer solution (pH 8.8) at 50 mM, along with 50 μL of 50 mM L-phenylalanine, and incubated in a water bath at 37°C for 20 min. The absorbance was measured at 280 nm.

2.4.3 β-1,3-Glucanases Activity

The activity of β-1,3-glucanases was evaluated using a colorimetric method as described in [35], with some modifications. 8 μL of enzymatic extract were added to 885 μL of a sodium acetate buffer solution (pH 5, 50 mM) along with 7 μL of laminarin at 0.15 wt%. The samples were incubated in a water bath at 40°C for 10 min, then placed in an ice bath for 5 min. Finally, 335 μL of 3,5-Dinitrosalicylic acid at 96 mM were added to the samples and incubated in a water bath at 90°C for 10 min, followed by another ice bath for 5 min. The absorbance was measured at 515 nm.

2.4.4 Chitinases Activity

Chitinase activity was evaluated following the method described by [37], with minor modifications. Briefly, the catalytic activity of chitinases in the presence of chitin as a substrate produces N-acetylglucosamine. To initiate the reaction, 5 μL of the enzymatic extract was added to 445 μL of sodium acetate buffer solution (pH 5) at 0.5 mM, along with 50 μL of chitin at 0.05 wt%. The solution was incubated at 40°C for 30 min, followed by the addition of 1 mL of potassium ferrocyanide, which was then kept in a water bath at 95°C for 15 min. To stop the reaction, the samples were placed in an ice bath. Absorbance was measured at 420 nm.

2.4.5 Peroxidase Activity (POD)

POD activity was conducted as described in [38], with minor modifications. A total of 33 μL of enzymatic extract was added to 1 mL of reaction media consisting of a sodium phosphate buffer solution (10 mM, pH 6), guaiacol (0.25 wt%), and hydrogen peroxide (10 mM). The absorbance was measured at 470 nm every 20 s for 1 min.

2.4.6 Catalase Activity (CAT)

CAT activity was performed according to [38], with minor modifications. An enzymatic extract of 11.11 μL was added to 1 mL of reaction media consisting of 1 M Tris-HCl (pH 8), 5 mM EDTA (ethylenediaminetetraacetic acid), and 30 mM. The absorbance was measured at 240 nm every 10 s for 1 min to monitor the CAT catalytic performance in the presence of H_2O_2.

2.5 Chlorophyll Fluorescence

Chlorophyll fluorescence measurements were recorded according to [39,40]. Using a chlorophyll meter, FluorPen FP 100 (Photon Systems Instruments, Czech Republic), measurements were taken from a dark-adapted second pair of leaves for 5 min. The data were recorded at 24, 48, and 72 h after COS foliar exposure, with four replicates for each treatment. Potential photochemical yield (Fv/Fm) values were extracted from OJIP curves.

2.6 Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$) with four replicates. Additionally, Principal Component Analysis (PCA) was performed for groups of COS and chitosan concentrations related to the studied enzymatic activities. R Studio was utilized for statistical analysis and to generate graphical representations.

3 Results and Discussion

Varieties of *Coffea arabica* and *Coffea canephora* have the highest economic impact on the global coffee indusdry. However, *Coffea arabica* plantations have faced biotic and abiotic stresses, such as climate change and disease advances concerning agricultural communities and coffee markets [41]. In response to these issues, it is essential to develop new sustainable technologies to alleviate the effects of biotic and abiotic stress factors in coffee plantations. This includes the synthesis and foliar application of chitosan oligosaccharides (COS) [21]. Chitosan and its derivatives are widely studied as elicitors of plant immune responses. Unfortunately, there is limited research and information regarding the potential application of COS in *Coffea arabica* plants.

3.1 Characterization of Chitosan Oligosaccharides

In this study, the microwave-assisted synthesis of COS was successfully conducted in the presence of acetic acid and hydrogen peroxide, according to the COS characterization results [20]. The synthesized COS exhibited an average molecular weight of 3547.90 ± 0.33 Da based on intrinsic viscosity values ($[\eta]$ = 23.3 ± 0.18 cP), a polymerization degree (PD) of 18.91 ± 0.0018, and a degree of deacetylation (DD%) of 76.64 ± 1.12, compared to chitosan's original maximum supplier values of 190,000 Da based on viscosity (300 cP) and a measured DD% of 76.67 ± 2.32. This illustrates the effect of chemical and physical hydrolysis on the viscosity and molecular weight of oligosaccharides, resulting in reductions of 92.23% ± 0.12 and 98.13% ± 0.00017, respectively, without influencing the degree of deacetylation (DD%) of 0.91% ± 0.97, which aligns with previously reported findings [19] due to the synthesis conditions that only favored the glycosidic bond rupture.

COS physicochemical properties define its bioactivity and impact on exposed plants [21,42]. Completely deacetylated COS is less capable of being recognized by plant receptors and activating the plant immune system [21]. The elicitor function of COS in activating plant defense mechanisms closely relates to PD. To enhance COS bioactivity, applied COS should have a PD of at least 4 [42].

Additionally, molecular weight and the method of application are key factors influencing the bioassimilation of chitosan and its derivatives. The molecular weight of the used chitin derivative influences its water solubility and viscosity, making it a vital factor in the mobilization and availability of COS within plant tissues [43,44].

The FTIR spectra of chitosan and chitosan oligosaccharides (Fig. 1) exhibit similar behavior, with changes in transmittance intensity corresponding to glycosidic bond breakage [19]. Both polysaccharides display chatacteristic peaks related to saccharide structures at 1151.3, 1060.7, 1018.5, and 895.81 cm^{-1} associated with C-O and β-1,4-glycosidic bond stretching vibrations [45–47]. The peak at 1660.2 cm^{-1} is linked to C-N vibration, while those at 1626, 1453, and 1420 cm^{-1} relate to amide I (C=O); the peak at 1320 cm^{-1} pertains to amide III [32,45,46,48]. Sharp bands at 1400.7 and 1376.5 cm^{-1} are assigned to C-H bending and stretching vibrations [49], and the absorption at 1251.8 cm^{-1} corresponds to C-O-C stretching vibrations. The peaks at 3200.6, 2861, and 2921 cm^{-1} are associated with -OH vibration and C-H stretching [48,49].

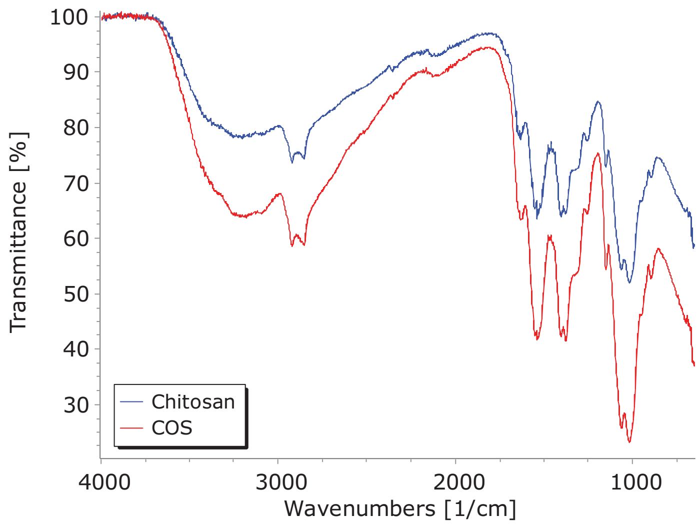

Figure 1: FTIR spectrum of chitosan and chitosan oligosaccharides (COS)

3.2 Enzymatic Activity

The primary mechanism involving elicitor molecules, such as chitosan and COS entails activating the plant's defense responses. In reaction to microbial invasion, plants undergo biochemical changes associated with biotic stress signaling, which modifies their physiology. For instance, available oxygen can be utilized to generate Reactive Oxygen Species (ROS), which inhibit pathogen growth [5,15]. Additional defense strategies encompass cell wall thickening, oxidative bursts, hypersensitive responses (HR), the synthesis of antimicrobial compounds like phenolics, flavonoids, and lignins, and the expression of antifungal pathogenesis-related proteins (PR) such as chitinases and glucanases [5,15–17].

The foliar application of chitosan has been well-documented to activate defense mechanisms associated with Systemic Acquired Resistance (SAR) responses. Chitin and its derivatives, such as chitosan and chitosan oligosaccharides, are recognized by plants as Microbial Associated Molecular Patterns (MAMPs) via transmembrane proteins called Pattern Recognition Receptors (PRRs) and the CERK1 receptor (Chitin Elicitor Receptor Kinase 1) [50]. The activation of defense mechanisms by exogenous elicitors like chitosan and chitosan oligosaccharides, which function as MAMPs, is linked to the expression of pathogenesis-related proteins (PR proteins), including β-1,3-glucanases and chitinases. These enzymes play a key rol fall under the category of PR proteins with catabolic activity; these enzymes degrade β-1,3-glucan and chitin in the fungal cell walls of pathogens, respectively [51].

Chitinases and β-1,3-glucanase activity lead to the release of low molecular weight saccharides, including oligosaccharides and polysaccharides, which are recognized by PRRs as elicitors or MAMPs. This recognition activates immune signaling pathways, triggering Pattern Triggered Immunity (PTI) [52–55]. The activation of PTI is associated with plant defense mechanisms, involving enzymatic pathways such as Phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), and peroxidases (POX) [15]. The overall plant defense response (both enzymatic and non-enzymatic) near the wound site includes the SAR mechanism [56].

Systemic Acquired Resitance SAR mechanisms can be triggered by external compounds that function as signaling molecules, MAMPs, or Damage-Associated Molecular Patterns (DAMPs). These include salicylic acid, jasmonic acid, polysaccharides, and oligosaccharides such as chitosan, alginate, and carrageenan, along with their oligosaccharide derivatives, to induce the expression of defense genes [17,18,57,58].

The foliar application of COS increases the total soluble protein content related to various metabolic processes induced by COS application; in contrast, chitosan decreases protein content compared to the control treatment. Additionally, chitosan increases total soluble protein content depending on the applied concentration. The accumulation of total soluble protein can be linked to cell redox homeostasis under induced stress for Reactive Oxygen Species (ROS) scavenging [59]. Notable, COS applications exhibits higher 1,3-glucanase activity than the control and chitosan treatments.

The differences in β-1,3-glucanase and chitinase activity suggest improved assimilation of the applied exogenous elicitor, COS, compared to other chitosan derivatives. PR proteins accumulate in the apoplast to inhibit the extracellular growth of pathogens. For instance, β-1,3-glucanase (PR2) and chitinases (PR3) break down penetration structures such as the intercellular hyphae and haustoria walls of phytopathogens by catalyzing the hydrolysis of chitin and β-glucans [16,53,58].

The results (Fig. 2) indicate a significant increase in soluble protein content (SPC) in *C. arabica* var. Borbon treated with COS at 0.25%, 0.75%, and 1%, compared to control plants treated with distilled water. In contrast, a notable reduction in SPC was observed in plants treated with Chitosan at 0.25% and 0.5%. Plants treated with COS at 0.5% and Chitosan at 0.5% did not display a significant difference compared to control plants ($p < 0.05$). All enzymatic activity results (Fig. 2) are expressed as specific activity based on SPC results.

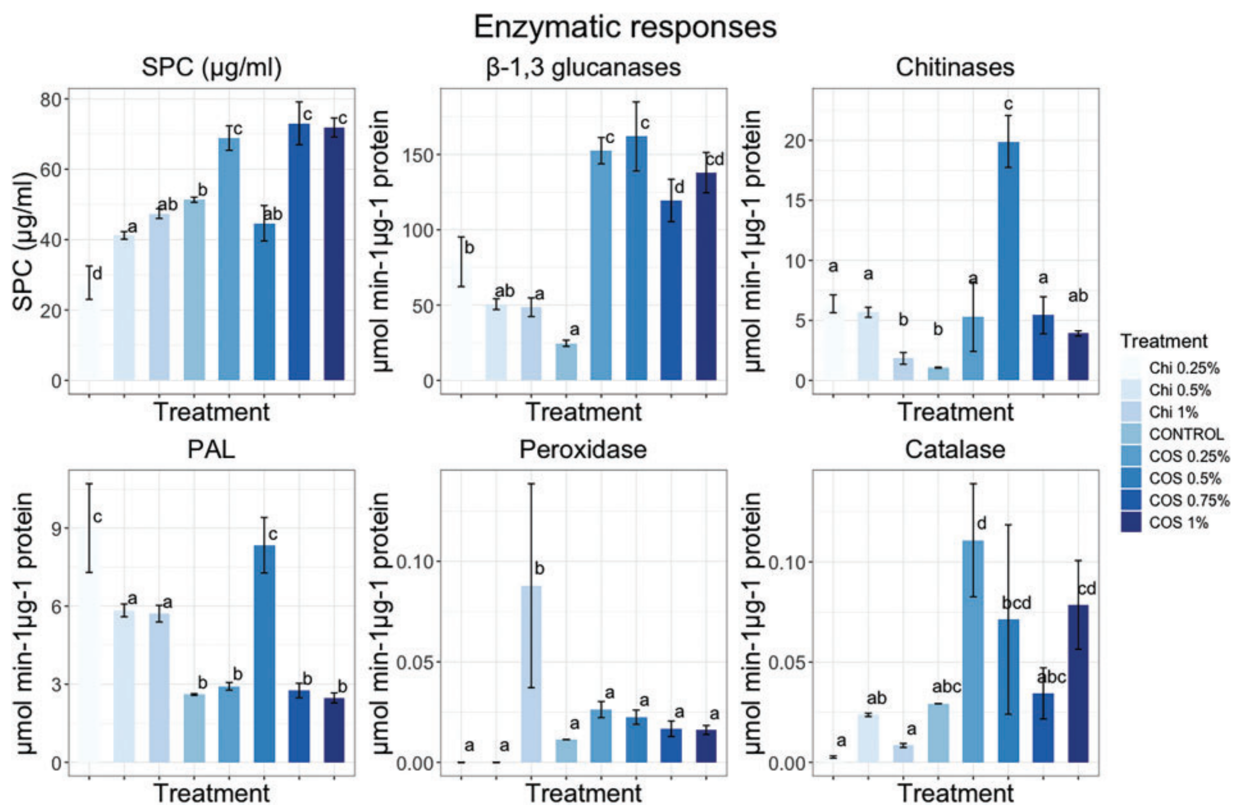

Figure 2: Enzymatic responses of *Coffea arabica* var. Bourbon to foliar exposure of chitosan, chitosan oligosaccharides, and distilled water used as a control. The evaluated specific responses were soluble protein content by Bradford methodology, β-1,3-glucanase, chitinases, PAL, peroxidases, and catalase

C. arabica var. Borbon plants treated with COS at concentrations of 0.25%, 0.5%, 0.75%, and 1%, along with chitosan at 0.25%, exhibit an increase in β-1,3-glucanase activity (Fig. 2) compared to control plants

($p < 0.05$). Conversely, plants treated with chitosan at 0.5% and 1%, which did not show a significant difference ($p < 0.05$). The plants treated with COS at 0.25% and 0.5% demonstrate the highest β-1,3-glucanase activity, as well as those treated with chitosan at 1%. The chitinase activity results (Fig. 2) indicate a significant increase ($p < 0.05$) in plants treated with COS at 0.25%, 0.5%, 0.75%, and 1% and chitosan at 0.25%, 0.5%, and 1% compared to control plants. The plants treated with COS at 0.5% display the highest chitinase activity.

Treatments with COS 0.5% and Chitosan 0.25% exhibited higher PAL activity (Fig. 2), followed by Chitosan 0.5% and 1%. In contrast, COS at 0.25%, 0.75%, and 1% did not show significant differences ($p < 0.05$) when compared to control plants. Moreover, only Chitosan 1% demonstrated a significant increase ($p < 0.05$) in POD activity (Fig. 2) compared to the control and plants treated with COS 0.25%, 0.5%, 0.75%, 1%, and Chitosan 0.25% and 0.5%. Higher CAT (Catalase) activity (Fig. 2) was observed in plants treated with COS 0.25%, while no significant differences ($p < 0.05$) were observed in plants treated with COS at 0.5%, 0.75%, 1%, or Chitosan at 0.25%, 0.5%, and 1%.

Phenylalanine ammonia-lyase (PAL) plays regulatory rol in the synthesis of phenylpropanoid compounds [21]. The increase in β-1,3-glucanase and chitinase activity has been associated with PAL activity; while, the rise of reactive oxygen species (ROS) at low concentrations, such as hydrogen peroxide (H_2O_2), acts as secondary messengers in the phenylpropanoid pathway [21,51]. The increase in PAL activity is linked to lignin synthesis, which strengthens plant cell walls. By engaging PAL in the synthesis of cinnamic acid from phenylalanine, the production of cinnamic acid contributes to the synthesis of phenolic compounds like lignin and flavonoids [21,43]. The results suggest a correlation between β-1,3-glucanases, chitinases, and antioxidant enzymes such as catalase (CAT) and peroxidase (POD) with PAL activity in plants treated with COS 0.5 wt%. The observed enzymatic activities suggest that COS 0.5 wt% treatment holds promise for enhancing SAR and ISR responses (Induced Systemic Resistance). Coffee plants treated with COS 0.5 wt% exhibit higher enzyme activity levels of β-1,3-glucanases, chitinases, POD, CAT, and PAL, which may correlate with a potentially better response and greater efficacy in controlling biotrophic pathogens [21,43,51].

Principal Component Analysis (PCA) results show that PC1 accounts for 41.3% of the variance, while PC2 explains 34.25% (Fig. 3). It demonstrates how the chitosan groups at 0.25%, 0.5%, and 1% are clustered together near the control (C) group, suggesting a certain similarity among them. The dispersed and clustered groups of COS at 0.25%, 0.75%, and 1% reveal specific differences between COS and chitosan. The enzyme activity vectors indicate that antioxidant enzymes, such as POD, for chitosan groups compared to COS, but show slight differences when contrasted with the control group. Furthermore, PAL activity exhibits more influence for chitosan groups as well as the COS 0.5% group. In contrast, the COS 0.25%, 0.75%, and 1% groups demonstrate higher influences in chitinases (CHI), β-1,3-glucanase (GLU), and catalase (CAT).

Overall, β-1,3-glucanase, chitinases, PAL, and CAT activities were significantly higher in COS plants treated compared to those treated with chitosan. These differences may be attributed to the variations in bioavailability between the molecules. The bioavailability of these molecules could be enhanced by the increased hydrophilicity of COS along with the substantial reduction in the viscosity of chitosan to COS, from 300 cP to 23.3 ± 0.18 cP. These changes may improve the mobility of the molecules and potentially enhance their bioavailability in coffee plants. Additionally, the reduction in the molecular weight of chitosan compared to COS improves the bioassimilation of the molecules, as demonstrated by the increase in enzymatic activity [43,44]. The variability in defense enzyme activity in coffee plants resulting from foliar exposure to COS and Chi treatments can be attributed to differences in molecular weight, demonstrating improved bioassimilation for COS treatments, particularly for COS 0.5 wt% with Mw of 3549.90 ± 0.33 Da compared with the Mw of Chi of 190,000 Da.

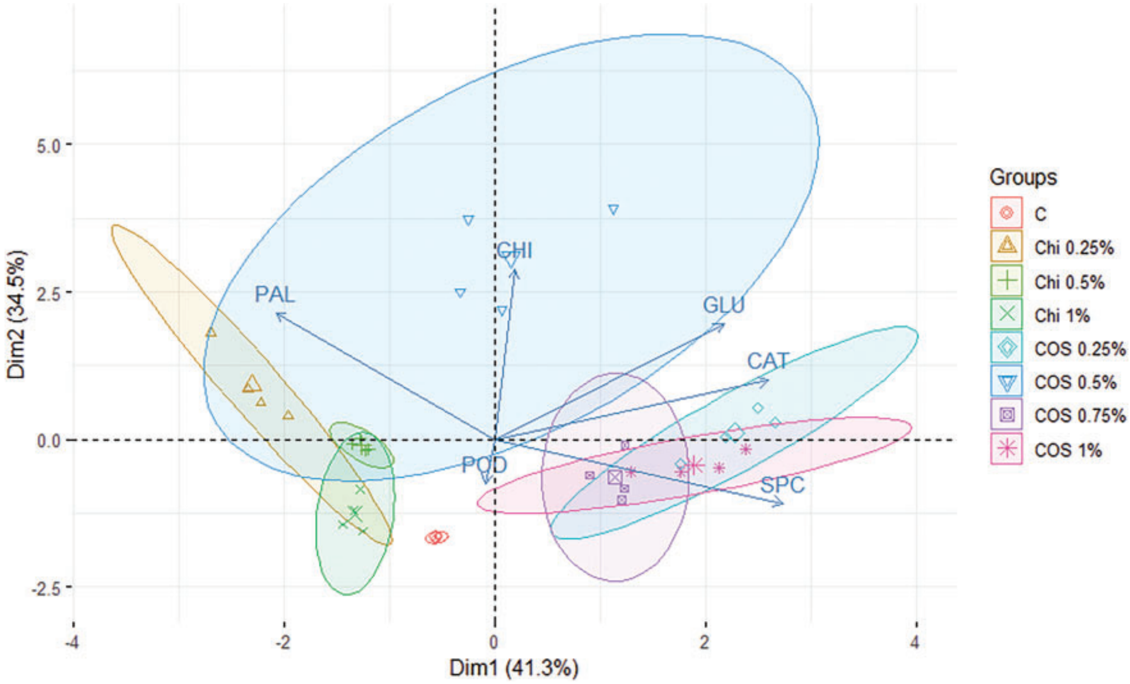

Figure 3: Principal Component Analysis (PCA) for enzymatic responses of *Coffea arabica* var. Borbon by exposure to chitosan (Chi) and chitosan oligosaccharides (COS) at different concentrations, compared to control C plants treated with distilled water

3.3 Chlorophyll Fluorescence

Chlorophyll fluorescence (Chl fluorescence) is a non-invasive technique used to measure the activity of PSII. It measures the fraction of absorbed light energy that is not utilized in photochemical reactions and is instead re-emitted as fluorescence. When a plant is exposed to photosynthetically active radiation (PAR), approximately 97% of this energy is utilized in photochemical reactions, while the remainder is released as fluorescence and heat [59,60]. Photochemical potential yield (Fv/Fm) values (Fig. 4) demonstrate slight differences between treatments at 24, 48, and 72 h after exposure. Twenty-four hours post-exposure, the Fv/Fm values indicate a minor decrease in COS 0.5%, but no treatment exhibited a significant difference ($p < 0.05$) from the control treatment. At 48 and 72 h after exposure, neither treatment displayed a significant difference ($p < 0.05$).

Fig. 4 suggests that COS and chitosan (Chi) exert a transient effect on the photochemical efficiency of PSII within the first 24 h following foliar application. A slight increase in Fv/Fm of PSII in the 0.75% and 1% treatments. This can be related to the increase in photopigments (chlorophyll a, b, and carotenoids) observed in the *Coffea canephora* var. Robusta assay with the foliar application of chitosan with 80% DD and 750,000 Da, along with COS with 2000 Da and DP 8–16 [61]. The increase in Fv/Fm may also be connected to photopigment concentrations and the impact of nutrient uptake from the treatments on the plants. This was suggested in an assay conducted on *Coffea canephora* Pierre Var. Robusta treated with chitosan nanoparticles (600 kDa, 85% DD) and chitosan oligomers, which could be linked to the property of chitosan derivatives to enhance magnesium and nitrogen levels in the leaves [23].

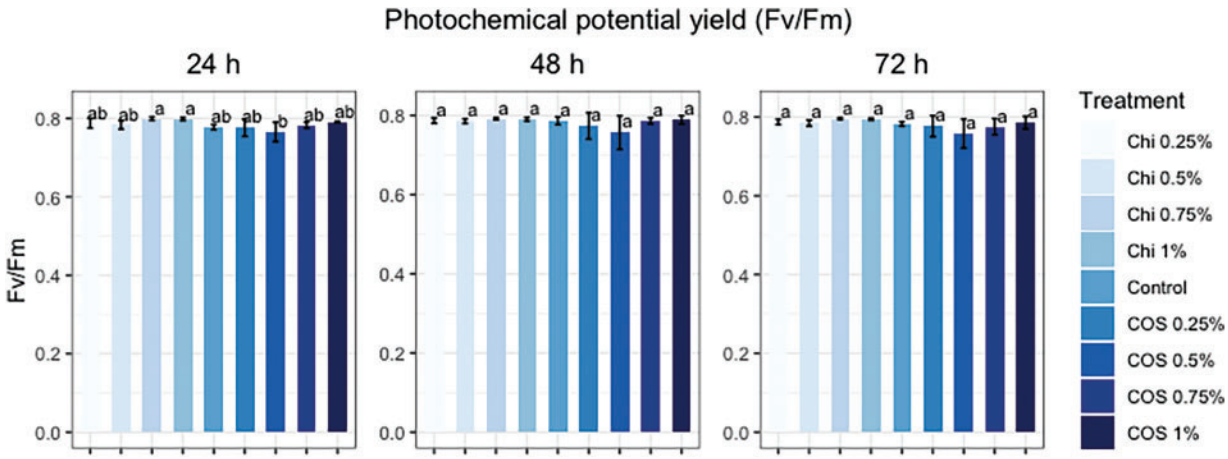

Figure 4: Photochemical potential yield (Fv/Fm) in *Coffea arabica* var. Borbon exposed to foliar applications of chitosan (Chi) and chitosan oligosaccharides (COS) at various concentrations compared to control plants treated with distilled water

4 Conclusions

The conducted study suggests the application of exogenous chitosan oligosaccharides (COS) is safe for *Coffea arabica* var. Borbon plants. Foliar application of COS with an average molecular weight of 3549.90 ± 0.33 and a Deacetylation Degree (DD%) of 76.64 ± 1.12 demonstrates a higher availability of amide I and III in FTIR spectrograms compared to chitosan samples. Thus, this can be associated with the increased specific enzymatic activity (β-1,3-glucanases, chitinases, PAL, and CAT) observed in coffee plants treated with COS. The COS treatment that yielded better responses was COS 0.5 wt%. However, coffee plants treated with chitosan exhibited a higher potential photochemical yield 24 h after application in comparison to both the control and COS treatments. Further research are needed to clarify the effect of foliar application of COS in coffee plants and its potential to mitigate the development of certain diseases like Coffee Leaf Rust (CLR).

Acknowledgement: The authors are grateful to the Universidad Autonoma de Baja California and Tecnologico Nacional de Mexico, Campus Tuxtla Gutierrez for the support provided to conduct this work. The authors also would like to express their gratitude to Consejo Nacional de Humanidades, Ciencias y Tecnologías de Mexico (Conahcyt).

Funding Statement: The authors received no specific funding for this study.

Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: Alexis Salazar-Navarro, Daniel González-Mendoza, Víctor Ruíz-Valdiviezo; data collection: José Joya-Dávila, Olivia Tzintzun-Camacho, Ulin Basilio-Cortes; analysis and interpretation of results: Dagoberto Duran-Hernández, Henry López-Lopez, Onecimo Grimaldo-Juárez; draft manuscript preparation: Alexis Salazar-Navarro, Daniel González-Mendoza, Víctor Ruíz-Valdiviezo. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

Abbreviations

CAT	Catalase
CERK1	Chitin Elicitor Receptor Kinase 1

CHI	Chitosan
Chl	Chlorophyll
CLR	Coffee Leaf Rust
COS	Chitosan oligosaccharides
CytPM-COS	Cytosinpeptidemycin and COS
DA	Degree of Acetylation
DAMPS	Damage Associated Molecular Patterns
DD	Deacetylation Degree
Fv/Fm	Potential photochemical yield
GlcN	D-glucosamine
GlcNAc	N-acetyl-D-glucosamine
HR	Hypersensitive Response
ISR	Induced Systemic Resistance
M_W	Molecular Weight average
PAL	Phenylalanine Ammonium Lyase
PD	Polymerization Degree
POX	Peroxidases
PPO	Polyphenol oxidase
PR	Pathogenesis Related proteins
PRRS	Pattern Recognition Receptors
PSII	Photosystem II
PTI	PAMPs or DAMPs triggered immunity
ROS	Reactive Oxygen Species
SAR	Systemic Acquired Resistance
SOD	Superoxide Dismutase
SPC	Soluble Protein Content

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CAPÍTULO 6. Estimulación bioquímica y fisiológica por nanopartículas de sílice en *Coffea arabica* var. *Bourbon*

Título original del manuscrito: Biochemical and Physiological Priming by Silica Nanoparticles Enhances Early Defense in Coffea arabica var. *Bourbon*

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Este capítulo corresponde a un manuscrito en preparación con fines de publicación en una revista especializada en biotecnología agrícola. En el momento de redacción de esta tesis, se encuentra considerado para su envío a la revista **Biocatalysis and Agricultural Biotechnology** *(Elsevier).*

Resumen

Este estudio evaluó los efectos bioquímicos y fisiológicos de nanopartículas de sílice (SiNPs) aplicadas foliarmente a plantas de *Coffea arabica* var. *Bourbon* bajo condiciones de invernadero. Se aplicaron concentraciones de 1.5, 3, 6 y 12 mM y se evaluaron parámetros como contenido de clorofila (SPAD), eficiencia fotosintética (Fv/Fm), fuga de electrolitos (EL), índice de estabilidad de membrana (MSI) y la actividad de enzimas relacionadas con defensa (PAL, quitinasa, β-1,3-glucanasa, catalasa y peroxidasa). Además, se realizaron ensayos con discos foliares inoculados con urediniosporas de *Hemileia vastatrix*. Se observó que concentraciones bajas (1.5–3 mM) inducen un estado de "priming" sin efectos fitotóxicos, mientras que concentraciones más altas activan vías de defensa dependientes de ácido salicílico. Las SiNPs redujeron la germinación de esporas y preservaron la integridad celular en tejidos tratados. Los resultados sugieren que las SiNPs podrían emplearse como agentes elicitadores sostenibles para la protección temprana contra roya del café.

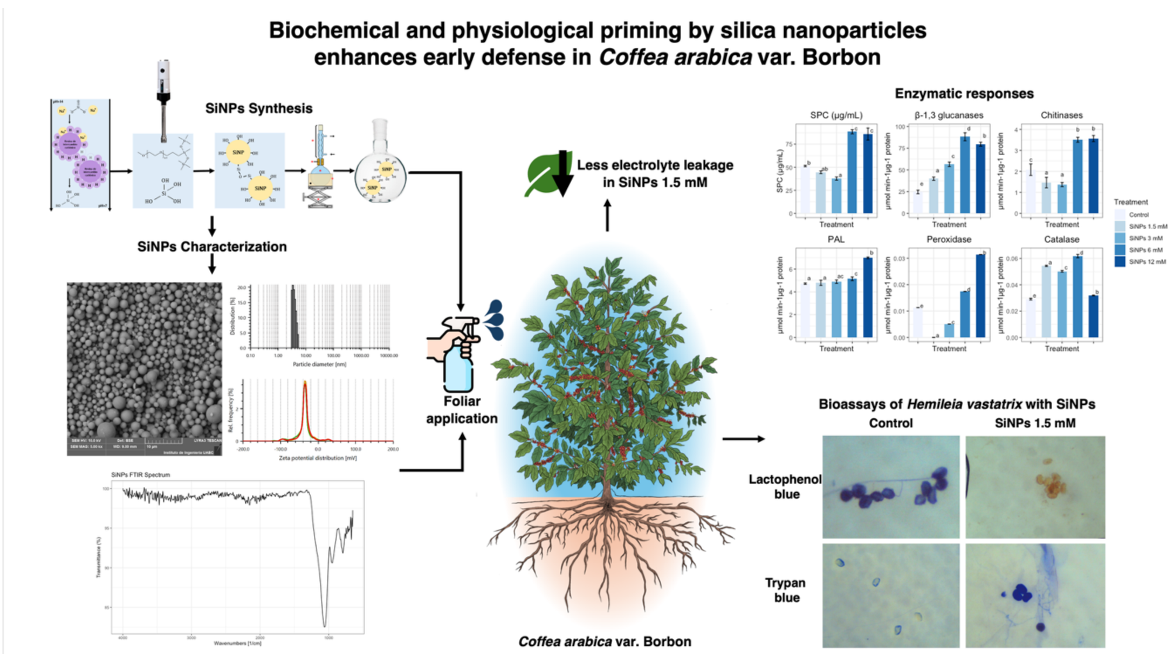
Palabras clave: nanopartículas de sílice, defensa inducida, priming, roya del café, *Coffea arabica*, elicitores foliares

Abstract

This study evaluates the biochemical and physiological responses of *Coffea arabica* var. *Bourbon* to foliar application of silica nanoparticles (SiNPs) at concentrations of 1.5, 3, 6, and 12 mM under greenhouse conditions. Treatments were arranged in a completely randomized design with four replicates, and analyses were performed 72 hours post-application. Biochemical parameters included chlorophyll content (SPAD), chlorophyll fluorescence (Fv/Fm, NPQ), electrolyte leakage (EL), and the activity of defense-related enzymes: phenylalanine ammonia-lyase (PAL), chitinase, β-1,3-glucanase, peroxidase, and catalase. The 6 and 12 mM SiNP treatments significantly enhanced enzymatic activity, particularly catalase, chitinase, and glucanase at 6 mM, and PAL and peroxidase at 12 mM, suggesting activation of salicylic acid–mediated defense pathways. The 1.5 mM treatment led to the lowest EL values and the highest membrane stability, indicating improved cell integrity without phytotoxic effects. Although fluorescence parameters did not differ significantly, plants treated with 1.5 and 3 mM SiNPs showed favorable trends in photosystem performance, including stable Fv/Fm and reduced NPQ. To our knowledge, this is the first report demonstrating biochemical evidence consistent with SiNP-induced priming in *Coffea arabica*, highlighting their potential as elicitors for enhancing crop resilience and supporting integrated disease management strategies.

Keywords: Induction of defense; Photosystem II; Enzymatic activity; Silica nanoparticles; nanoprotection of plants; Foliar elicitors

Graphical abstract



1. Introduction

Nanoparticles (NPs), typically ranging from 1 to 100 nm in diameter, exhibit distinct physicochemical properties that enhance their reactivity and biological efficacy compared to their bulk counterparts. These include a high surface area-to-volume ratio, tunable surface chemistry, and improved interaction with biological systems. Depending on their composition, NPs can be classified as metallic, organic, or composite, and are synthesized via bottom-up or top-down approaches (Kale et al., 2024; Muhammad et al., 2022; Munir et al., 2023). In agriculture, nanomaterials have emerged as sustainable alternatives to conventional agrochemicals, offering enhanced delivery, reduced environmental impact, and potential biostimulant or elicitor activity (Naidu et al., 2023; Weisany et al., 2024).

Silica nanoparticles (SiNPs), in particular, are promising due to their biocompatibility, structural versatility, and capacity to alleviate both abiotic and biotic stress. They are often synthesized via the Sol-Gel method using precursors such as tetraethyl orthosilicate (TEOS) or sodium metasilicate, producing amorphous SiNPs capable of releasing bioavailable silicic acid (Salazar-Navarro and Salas-Valdez, 2022; Yadav et al., 2023). Once applied, SiNPs can penetrate plant tissues through cuticles or stomata and are internalized via passive or active mechanisms depending on size, polarity, and pH (Souri et al., 2021; Wang et al., 2022). After absorption, silicon is translocated and deposited as silica gel in epidermal tissues, where it reinforces mechanical resistance and contributes to water-use efficiency (Domenico Prisa, 2023).

Beyond physical protection, SiNPs play a regulatory role in plant immunity by stimulating the salicylic acid (SA) signaling pathway, enhancing the biosynthesis of phenolics and lignin, and inducing defense-related enzymes such as phenylalanine ammonia-lyase (PAL), chitinase, β-1,3-glucanase, peroxidase, and catalase (Du et al., 2022; Weisany et al., 2024). These biochemical responses have been linked to systemic acquired resistance (SAR), positioning SiNPs as potential elicitors in integrated disease management programs.

Coffee (*Coffea arabica*), one of the world's most valuable crops, is increasingly threatened by climate-driven stressors, soil nutrient depletion, and phytopathogens such as *Hemileia vastatrix*, the causal agent of coffee leaf rust (CLR), and *Hypothenemus hampei* (coffee berry borer) (Ayalew et al., 2024; Paragon Ritonga and Kwon, 2024). These challenges are particularly severe in Latin America, where Mexico is among the top producers of *C. arabica*, with key cultivation areas in Chiapas, Veracruz, and Puebla (Gabriel-Hernández and Barradas, 2024).

Although silicon has been widely studied for stress mitigation in crops, the biochemical and physiological effects of foliar-applied SiNPs in *C. arabica* remain underexplored. This study evaluates the impact of SiNPs synthesized via the Sol-Gel method on *C. arabica* var. *Bourbon* under greenhouse conditions. In addition to assessing enzyme activity, membrane stability, and photochemical efficiency, we incorporated infection assays using leaf disks inoculated with *H. vastatrix*. These include histochemical staining techniques and measurements of electrolyte leakage (EL) and membrane stability index (MSI) to evaluate early host-pathogen interactions. The findings aim to clarify the elicitor potential of SiNPs and their role in activating plant defense without inducing phytotoxicity.

2. Materials and Methods

2.1. Synthesis of Silica Nanoparticles (SiNPs)

SiNPs were synthesized following the Sol-Gel methodology described by Salazar-Navarro et al. (Salazar-Navarro et al., 2023; Salazar-Navarro and Salas-Valdez, 2022). A 100 mL stock solution of 0.3 M sodium metasilicate pentahydrate was filtered through 25 g of ion exchange resin. Subsequently, 5 mL of ethanol was added dropwise, and the mixture was homogenized at 2000 rpm with 200 mL of a 1% Silwet™ L-77 (Momentive®) solution for 30 min. The resulting dispersion was refluxed at 105 °C for 30 min.

2.2. Characterization of SiNPs

The hydrodynamic diameter and zeta potential of the SiNP suspension were measured via dynamic light scattering (DLS) in distilled water using an Anton Paar®

Litesizer 500 particle analyzer. To assess dry particle size, the suspension was dried at 70 °C for 6 h. Elemental composition and morphology were analyzed using a JEOL® JSM-601LA scanning electron microscope (SEM) equipped with an energy-dispersive X-ray detector (EDX) and backscattered electron detector (BSE) at 200× and 15,000× magnification under 10 kV.

2.3. Plant Material and Treatments

Six-month-old *Coffea arabica* var. *Bourbon* plants were cultivated in polyethylene bags (6.7 × 6 × 26 cm) filled with a peat moss:agrolite (3:1) substrate under greenhouse conditions. Plants were irrigated twice weekly with 100 mL of potable water (Salazar-Navarro et al., 2025). For biochemical assays, foliar treatments consisted of spraying 2 mL of silicon nanoparticles (SiNPs) at concentrations of 1.5, 3, 6, or 12 mM. Distilled water was used as a control. Treatments were applied 72 hours prior to tissue sampling. Each treatment included four biological replicates.

2.4. Chlorophyll Fluorescence

Chlorophyll fluorescence and non-photochemical quenching (NPQ) were measured at 24, 48, and 72 h after treatment using a FluorPen FP 100 fluorometer (Photon Systems Instruments). Measurements were performed on the second leaf pair after 5 min of dark adaptation. The OJIP test used a light intensity of 3000 µmol photons m^{-2} s^{-1}. NPQ was assessed using five 7-s light pulses at 12-s intervals and three 1-s dark pulses at 26-s intervals. The quantum yield of photosystem II (ΦPSII) was calculated as:

$$\Phi PSII = \frac{Fv}{F_m} \tag{1}$$

where *Fv* is variable fluorescence and *Fm* is maximum fluorescence (Moreno et al., 2008).

2.5. Enzymatic Activity Assays

Samples were collected 72 h after treatment from the second pair of leaves. Leaf tissue (50 mg) was ground in liquid nitrogen and macerated at 4 °C in an extraction buffer containing 0.1 M Tris-HCl (pH 8), 0.1% ascorbic acid, 10% glycerol, 1% PVP, and 5% β-mercaptoethanol. The extracts were centrifuged at 15,000×g for 15 min, and the supernatant was stored at –4 °C. Enzymatic activities were measured using a Nanodrop One® spectrophotometer (Thermo Fisher Scientific) as follows:

- Phenylalanine Ammonia-Lyase (PAL): 5 μL of protein extract was incubated with 145 μL of 50 mM Tris-HCl (pH 8.8) and 50 μL of 50 mM L-phenylalanine. Absorbance was read at 280 nm after 20 min at 37 °C (Reichel et al., 2022).
- β-1,3-Glucanase: 8 μL of extract was mixed with 885 μL of 50 mM sodium acetate (pH 5) and 7 μL of 0.15% laminarin. Samples were incubated at 40 °C for 10 min, then reacted with 335 μL of 96 mM DNS and heated at 90 °C for 10 min. Absorbance was measured at 515 nm (Luján-Hidalgo et al., 2020).
- Chitinase: 5 μL of extract was incubated with 445 μL of 0.5 mM sodium acetate buffer (pH 5) and 50 μL of 0.05% chitin. After 30 min at 40 °C, 1 mL of potassium ferrocyanide was added and heated at 95 °C for 15 min. Absorbance was read at 420 nm (Castro et al., 2011).
- Peroxidase (POD): 33 μL of extract was added to 1 mL of 10 mM sodium phosphate buffer (pH 6) containing 0.25% guaiacol and 10 mM H_2O_2. Absorbance was monitored at 470 nm every 20 s for 1 min (Santos-Espinoza et al., 2021).
- Catalase (CAT): 11.11 μL of extract was mixed with 1 mL of 1 M Tris-HCl (pH 8), 5 mM EDTA, and 30 mM H_2O_2. Absorbance was measured at 240 nm every 10 s for 1 min (Santos-Espinoza et al., 2021).

2.6. Electrolyte Leakage and Membrane Stability Index

Electrolyte leakage (EL) and membrane stability index (MSI) were determined following Trejo-Paniagua et al. (Trejo-Paniagua et al., 2024) with modifications. Leaf disks (10 mm diameter) were immersed in 15 mL of triple-distilled water and incubated at 25 °C for 2 h. Initial conductivity (EC_1) was measured. Samples were autoclaved at 121 °C and 15 psi for 15 min before measuring final conductivity (EC_2). EL and MSI were calculated using:

$$EL(\%) = \left(\frac{EC_1}{EC_2}\right) * 100 \quad (2)$$

$$MSI(\%) = \left(1 - \frac{EC_1}{EC_2}\right) * 100 \quad (3)$$

In addition, the same assay was applied to leaf disks inoculated with *H. vastatrix* (see Section 2.8) to evaluate the physiological response under biotic stress conditions. Disks were collected at 24, 48, and 72 h after inoculation, and EL/MSI values were measured as described above.

2.7. *Hemileia vastatrix* Spores Collection

Hemileia vastatrix urediniospores were collected from symptomatic coffee plants in a farm located in Ángel Albino Corzo, Chiapas, Mexico (15°45'33.8"N, 92°38'03.3"W). Infected leaves exhibiting active sporulation were harvested and transported to the laboratory. Urediniospores were gently scraped from the abaxial surface using a sterile scalpel and then passed through a 300 μm sieve to remove debris (Barquero-Miranda et al., 2023). The spores were suspended in sterile distilled water containing 0.5% (v/v) Tween 20 (Sigma-Aldrich®, USA) to prepare an inoculum at a final concentration of 1×10^4 spores mL^{-1} (Rojas-Chacón et al., 2023; Salcedo-Sarmiento et al., 2021).

2.8. Infection Assay, Foliar Disk Preparation and Microscopy

To evaluate early interactions between *Coffea arabica* and *Hemileia vastatrix* under controlled conditions, a qualitative infection assay was performed using foliar disks. Fully expanded leaves from the second and third node of six-month-old plants were selected. Leaf disks (10 mm in diameter) were excised using a sterile copper punch and surface-disinfected by immersion in 2% sodium hypochlorite for 1 minute, followed by two rinses in sterile distilled water (2 minutes each) under gentle agitation (Rojas-Chacón et al., 2023). The disks were dried using sterile paper towels and placed in sterile plastic boxes containing a 1 cm layer of polyester wadding moistened with 3 mL of distilled water to maintain high humidity.

Disks were then immersed for 30 seconds in either 1.5 mM SiNPs or sterile distilled water (control) (He et al., 2022; Hoang et al., 2022). After 24 hours of incubation at room temperature, each disk was inoculated with 10 µL of a suspension of *H. vastatrix* urediniospores (1 × 10^4 spores mL^{-1} in 0.5% Tween 20). The boxes were incubated at 22 °C in complete darkness for 24 hours (Barquero-Miranda et al., 2023; Várzea et al., 2023).

Spore germination and fungal development were evaluated at 24 and 48 hours post-inoculation using foliar imprint techniques. A thin layer of clear nail polish was applied to the abaxial surface of each inoculated disk. Once dried (approximately 1 minute near a Bunsen burner), the film was gently peeled off using fine tweezers to obtain the imprint. Each imprint was placed on a microscope slide and stained with 10 µL of either lactophenol blue (to visualize fungal structures) or trypan blue (to assess spore viability). A coverslip was then applied (Julca-Otiniano et al., 2024; Pathoumthong et al., 2023; Ramiro et al., 2009).

Samples were examined under a compound light microscope (AmScope, USA) equipped with a digital camera (AmScope MD35, USA) to assess differences in urediniospore germination, fungal structure development, and spore viability between control and SiNP-treated tissues.

The 1.5 mM concentration was selected for these assays based on its favorable performance in preliminary physiological assessments, including the lowest electrolyte leakage, highest membrane stability, and absence of negative effects on chlorophyll content or photosynthetic efficiency (SPAD, Fv/Fm, NPQ). This dose was considered optimal for evaluating plant–pathogen interactions under non-phytotoxic, defense-inducing conditions.

2.9. Statistical Analysis

Data were analyzed using one-way ANOVA, and means were compared using Tukey's test at $\alpha = 0.05$. All statistical analyses and visualizations were performed using RStudio (version 2025.05.0+496, R Foundation).

3. Results and Discussion

3.1. Characterization of SiNPs

Dynamic light scattering (DLS) analysis showed that the silica nanoparticles (SiNPs) synthesized via the Sol-Gel method exhibited an average hydrodynamic diameter of 3.98 nm, with a narrow polydispersity index (PDI) of 8.7%, and a highly negative zeta potential of –34.4 mV (Figure 1A–B). The electrical conductivity of the colloidal suspension was measured at 2.327 mS/cm.

These physicochemical parameters are indicative of a monodisperse and electrostatically stable nanoparticle suspension. The low PDI value (< 0.1) reflects uniform particle size distribution, a desirable characteristic for consistent interaction with biological tissues (Zhang and Wang, 2023). Furthermore, the high negative zeta potential suggests strong electrostatic repulsion among particles, which prevents aggregation and contributes to long-term colloidal stability. According to previous studies, zeta potential values beyond ±30 mV are typically associated with stable dispersions in aqueous systems (Öztürk et al., 2024).

The small hydrodynamic diameter observed in this study falls within the range required for efficient foliar uptake, particularly through stomatal or cuticular pathways. Nanoparticles smaller than 20 nm are more likely to translocate across cell walls or epidermal barriers via passive diffusion or endocytic-like mechanisms (Azim et al., 2023; Wang et al., 2016; Wu and Li, 2022). Thus, the nanometric scale and physicochemical uniformity of these SiNPs support their suitability for foliar application in agricultural systems. The DLS analysis confirms that the SiNPs are adequately small, stable, and dispersed to ensure effective interaction with plant tissues, minimizing the risk of aggregation or phytotoxicity upon application.

Scanning electron microscopy (SEM) analysis revealed that the dried SiNPs exhibited a predominantly spherical morphology, with diameters ranging from 694 to

2767 nm (Figure 2A–C). This substantial increase in size, relative to the hydrated nanoparticles measured by DLS, is likely due to agglomeration and partial fusion that occurred during the drying process. These effects are typical of nanoparticles synthesized via the Sol-Gel method, where the condensation of silanol groups (Si–OH) under reduced water availability and increased ionic strength promotes secondary aggregation (Owoeye et al., 2021a; Rahman and Padavettan, 2012; Salazar-Navarro and Salas-Valdez, 2022; Stober et al., 1968).

The spherical shape and relatively smooth surface of the particles, along with the absence of crystalline facets, indicate an amorphous structure—a common result when using sodium metasilicate as a precursor under aqueous and moderate temperature conditions (Owoeye et al., 2021a). This amorphous character is particularly desirable for agricultural applications, as it favors gradual degradation, release of bioavailable silicic acid, and biocompatibility with plant tissues (El-Shetehy et al., 2021; Mukarram et al., 2024; Yan et al., 2024).

Elemental mapping by energy-dispersive X-ray spectroscopy (EDX) (Figure 2D–E) confirmed silicon (Si) and oxygen (O) as the primary constituents, corresponding to the formation of siloxane (Si–O–Si) and silanol (Si–OH) functional groups in the silica network. The presence of carbon (C) was attributed to residual Silwet™ L-77, a non-ionic surfactant used during synthesis to stabilize the colloidal dispersion and delay gelation.

Traces of sodium (Na) may also persist due to the use of sodium metasilicate as the silica precursor; however, these were effectively reduced through cation exchange using an ion-exchange resin during the synthesis process. This step is critical for lowering the concentration of electrolytes in the reaction medium, thereby slowing the gelation rate and allowing better control over the Sol-Gel kinetics (Akkaya et al., 2024; AlMohaimadi et al., 2024; Jeffrey Brinker, 2005; Salazar-Navarro and Salas-Valdez, 2022).

High electrolyte concentrations are often associated with rapid, disordered 3D condensation of silanol groups, leading to the formation of irregular, unstable aggregates. In contrast, reducing ionic strength promotes the formation of well-defined, homogeneous, and morphologically stable structures, resulting in spherical and monodisperse nanoparticles (Jeffrey Brinker, 2005; Salazar-Navarro and Salas-Valdez, 2022). Thus, cation exchange not only enhances the chemical purity of the SiNPs but also favors structural integrity and colloidal stability, both essential attributes for their use in biological and agricultural applications.

The uniform elemental distribution observed in the EDX mapping supports the successful synthesis of chemically homogeneous SiNPs, free from contamination by potentially phytotoxic metal ions. This purity is critical in biological and agricultural contexts, where consistency, non-toxicity, and predictable behavior are essential for safe and effective foliar application.

Fourier-transform infrared spectroscopy (FTIR) provided further confirmation of the chemical structure and functional groups present in the synthesized SiNPs. The spectrum exhibited prominent bands corresponding to the asymmetric and symmetric stretching vibrations of siloxane (Si–O–Si) bonds, centered at approximately 1097.7 cm^{-1} and 799.5 cm^{-1}, respectively. These peaks are characteristic of a polymerized silica network and reflect the presence of a highly cross-linked siloxane framework, typical of materials synthesized via Sol-Gel processes (Liang et al., 2023; Maryam et al., 2024; Owoeye et al., 2021b)

A distinct absorption band at 948.6 cm^{-1} was assigned to the symmetric stretching of silanol (Si–OH) groups, which are indicative of surface hydroxylation. These –OH groups are crucial for colloidal stability, aqueous dispersion, and interaction with biological membranes, and may contribute to the bioavailability of silicic acid upon nanoparticle degradation (Maryam et al., 2024).

Additionally, a bending vibration observed at 670.9 cm^{-1} was also associated with Si–O–Si linkages and further supports the formation of an amorphous silica matrix (Krivoshein et al., 2022). The absence of peaks corresponding to other chemical species (e.g., nitrates, carbonates, or phosphates) suggests a high chemical purity of the synthesized SiNPs, in line with the elemental findings from EDX.

From an agricultural perspective, the presence of surface silanol groups enhances the potential biocompatibility and reactivity of the SiNPs with plant tissues, facilitating controlled release of orthosilicic acid (H_4SiO_4)—the plant-available form of silicon (Li et al., 2025; Naidu et al., 2023; Sarkar et al., 2022). These structural features are especially relevant for nanoparticle-mediated priming strategies aiming to stimulate plant defense responses without introducing toxic residues.

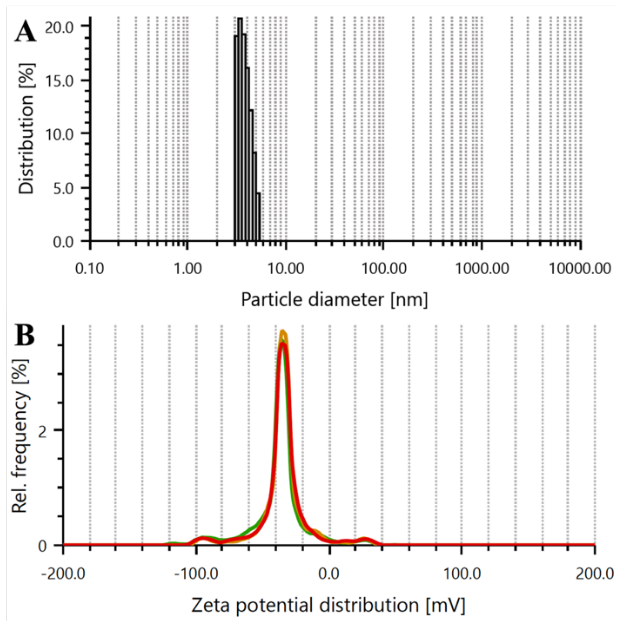


Figure 1. DLS characterization of silica nanoparticles (SiNPs). (A) Particle size distribution of SiNPs suspended in distilled water showing a mean hydrodynamic diameter of 3.98 nm. (B) Zeta potential distribution indicating a peak around –34.4 mV, suggesting good colloidal stability.

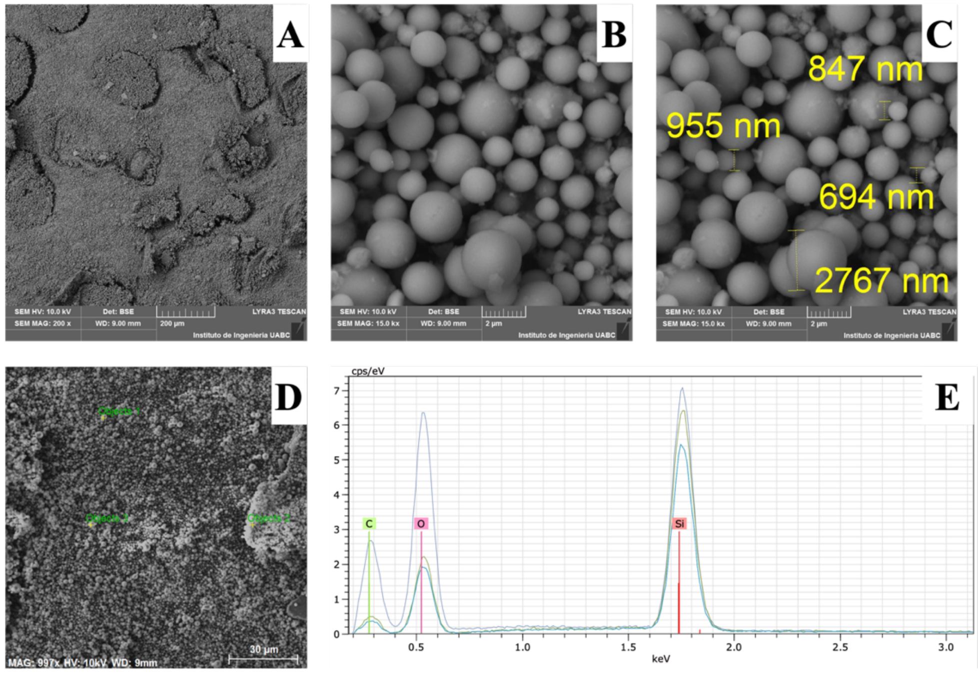


Figure 2. SEM and EDX analysis of silica nanoparticles (SiNPs). (A) SEM image at 200× magnification showing agglomerated morphology of dried SiNPs. (B) SEM image at 15,000× magnification showing spherical nanoparticle morphology. (C) Particle size annotations showing SiNPs ranging from 694 nm to 2767 nm in diameter. (D) SEM image with EDX mapping points for elemental composition. (E) EDX spectrum confirming the presence of silicon (Si) and oxygen (O), with minor carbon (C) signals from surfactant residues.

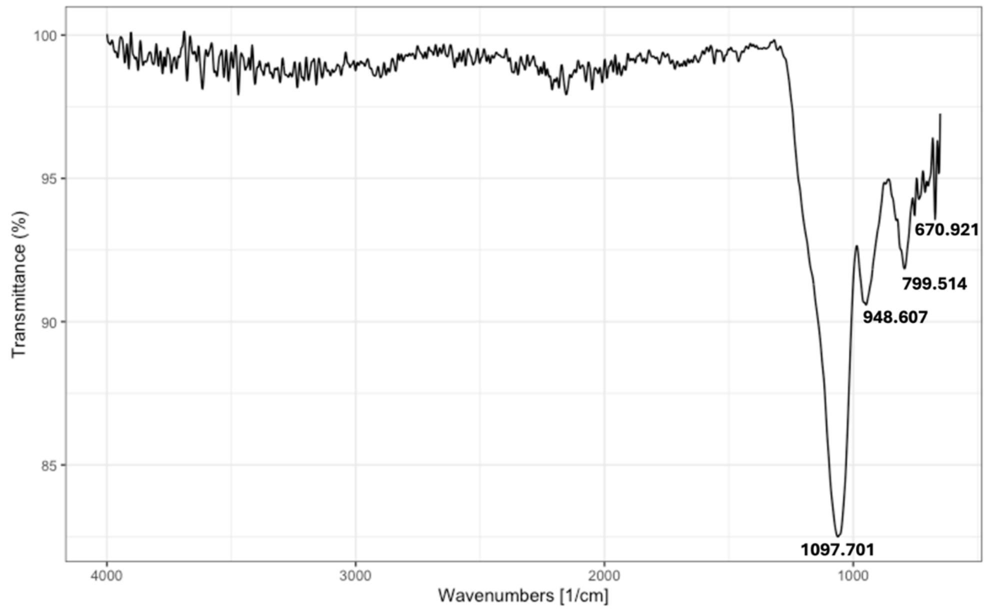


Figure 3. FTIR spectrum of silica nanoparticles (SiNPs). Spectrum showing characteristic vibrational bands of Si–O–Si and Si–OH groups. Notable peaks include asymmetric stretching of Si–O–Si at 1097.7 cm^{-1}, symmetric stretching at 799.5 cm^{-1}, symmetric Si–OH stretch at 948.6 cm^{-1}, and Si–O–Si bending at 670.9 cm^{-1}.

3.2. Photosynthetic Parameters (Fv/Fm, NPQ, SPAD)

The photochemical efficiency of photosystem II (Fv/Fm) in *Coffea arabica* var. *Bourbon* remained within the optimal range (~0.77–0.78) over the 72-hour evaluation period in the control and in plants treated with 1.5 and 3 mM SiNPs (Table 1). These values are consistent with healthy, non-stressed plants, suggesting that low to moderate SiNP concentrations do not induce photoinhibition (Larkunthod et al., 2022). In contrast, treatments with 6 and 12 mM SiNPs caused a progressive decline in Fv/Fm, reaching 0.64 in the 12 mM group at 72 h. This drop indicates potential damage to the photosynthetic apparatus, possibly resulting from oxidative stress or impaired energy conversion in the PSII complex (Sharf-Eldin et al., 2023; Siddiqui et al., 2020).

Non-photochemical quenching (NPQ), a key photoprotective mechanism that dissipates excess excitation energy as heat, showed an opposite trend. While NPQ values remained relatively stable in the control and low-concentration treatments (0.82–0.94), they increased significantly in the 12 mM treatment, reaching 1.00. Elevated NPQ suggests the activation of protective responses to prevent overexcitation of PSII under stress conditions (Liang et al., 2023), and may reflect a compensation mechanism in response to SiNP-induced oxidative pressure.

SPAD values, representing relative chlorophyll content, showed no significant changes at 1.5 and 3 mM SiNPs, further supporting the absence of pigment degradation at these concentrations (Shah et al., 2017). However, a mild yet significant reduction in SPAD was observed at 12 mM, reinforcing the interpretation of stress at higher nanoparticle doses.

From a physiological standpoint, the maintenance of Fv/Fm and SPAD values at 1.5–3 mM suggests that SiNPs may exert a priming effect, enhancing photosynthetic resilience without disrupting pigment biosynthesis or energy flow. Moreover, although silica itself is not directly involved in photosynthesis, its accumulation in mesophyll and epidermal tissues may indirectly enhance photosynthetic performance. For instance, silica deposition around chloroplast-containing cells has been associated with improved water retention, reduced transpiration, and modulation of ROS homeostasis, all of which contribute to sustained photochemical efficiency under stress (Mandlik et al., 2020; Souri et al., 2021).

Additionally, it has been proposed that Si-induced reinforcement of cellular membranes may stabilize the thylakoid structure under oxidative conditions, thereby protecting photosystems from lipid peroxidation and improving energy use efficiency (Du et al., 2022; Thakral et al., 2021). In this context, low-dose SiNP application could facilitate protective deposition patterns or serve as a slow-release source of bioavailable silicic acid, enhancing cellular tolerance mechanisms while preserving photosynthetic function.

Collectively, the decline in Fv/Fm and rise in NPQ at higher doses underscores the dose-dependent effect of SiNPs. While moderate concentrations promote photochemical stability and stress tolerance, excessive doses may interfere with electron transport and pigment stability. These findings support the importance of SiNP concentration optimization and highlight the potential of Si-based nanomaterials in enhancing crop physiological performance under controlled conditions.

Table 1. Photosynthetic and pigment-related parameters (Fv/Fm, NPQ, and SPAD) in *Coffea arabica* var. *Bourbon* after foliar application of SiNPs at different concentrations, measured at 24, 48, and 72 h.

Variable	Control	SiNPs 1.5 mM	SiNPs 3 mM	SiNPs 6 mM	SiNPs 12 mM
Fv/Fm 24 h	0.77 ± 0.01^{a}	0.78 ± 0.01^{a}	0.78 ± 0.01^{a}	0.75 ± 0.04^{a}	0.71 ± 0.07^{a}
Fv/Fm 48 h	0.79 ± 0.00^{a}	0.78 ± 0.00^{a}	0.78 ± 0.03^{a}	0.74 ± 0.06^{a}	0.69 ± 0.09^{a}
Fv/Fm 72 h	0.78 ± 0.00^{a}	0.78 ± 0.00^{a}	0.76 ± 0.04^{a}	0.74 ± 0.05^{a}	0.64 ± 0.13^{a}
NPQ 24 h	0.82 ± 0.17^{a}	0.83 ± 0.09^{a}	0.82 ± 0.07^{a}	0.91 ± 0.05^{a}	0.91 ± 0.24^{a}
NPQ 48 h	0.84 ± 0.07^{a}	0.86 ± 0.07^{a}	0.92 ± 0.12^{a}	0.92 ± 0.08^{a}	1.00 ± 0.10^{a}
NPQ 72 h	0.88 ± 0.11^{a}	0.94 ± 0.06^{a}	0.94 ± 0.11^{a}	0.98 ± 0.08^{a}	0.96 ± 0.17^{a}
SPAD 24 h	63.85 ± 2.76^{a}	62.33 ± 7.03^{a}	58.13 ± 6.49^{a}	58.13 ± 6.51^{a}	60.75 ± 7.94^{a}
SPAD 48 h	59.13 ± 4.8^{a}	62.4 ± 4.29^{a}	57.05 ± 5.18^{a}	59.95 ± 5.48^{a}	58.32 ± 7.67^{a}
SPAD 72 h	64.43 ± 1.57^{a}	63.67 ± 7.92^{a}	59.22 ± 5.8^{a}	60.65 ± 2.46^{a}	63.25 ± 3.15^{a}

Data represent mean ± standard deviation (n = 4). SiNPs: silica nanoparticles; Fv/Fm: photochemical efficiency of PSII; NPQ: non-photochemical quenching; SPAD: Soil Plant Analysis Development index. Different letters indicate statistically significant differences between treatments according to Tukey's HSD test (α = 0.05).

3.3. Soluble Protein Content and Stress Signaling

Figure 4 summarizes the biochemical responses of *Coffea arabica* var. *Bourbon* following foliar application of SiNPs. Soluble protein content (SPC) exhibited a dose-dependent trend. While plants treated with high concentrations (6 and 12 mM) showed a significant increase in SPC—suggesting metabolic activation and stress-induced protein biosynthesis—those treated with 1.5 and 3 mM SiNPs displayed slightly lower protein levels than the control. This decrease may reflect an early adaptive response, in which resources are redirected toward defense signaling and regulatory mechanisms rather than the accumulation of structural proteins. Similar patterns have been reported in plants undergoing moderate stress or priming events, where protein turnover increases without a net gain in total soluble protein (Emamverdian et al., 2020; Sarkar et al., 2024; Tofighi Alikhani et al., 2021).

Importantly, the stability of SPC and lack of protein overaccumulation at 1.5 and 3 mM, together with favorable photochemical parameters (see Section 3.2), suggest that these concentrations do not induce metabolic stress and may instead promote a primed physiological state. These findings support the rationale for selecting 1.5 mM SiNPs for subsequent inoculation assays in foliar disks, as this concentration combines low phytotoxicity with the potential to activate early defense responses.

3.4. Induction of Defense-Related Enzymes

Key pathogenesis-related enzymes—β-1,3-glucanases, chitinases, and phenylalanine ammonia-lyase (PAL)—were significantly induced in response to foliar SiNP application, particularly at 6 and 12 mM. Moderate increases in β-1,3-glucanase and chitinase activity were also observed at 3 mM, suggesting that even submaximal doses can partially activate basal defense mechanisms. These enzymes play a central role in plant immunity: β-1,3-glucanases and chitinases degrade fungal cell wall components, weakening the structural integrity of invading pathogens, while PAL catalyzes the first step of the phenylpropanoid pathway by deaminating L-phenylalanine to trans-cinnamic acid, a precursor of lignin, flavonoids, and a broad spectrum of antimicrobial phenolic compounds (Goswami et al., 2022; Saberi Riseh et al., 2023).

The upregulation of PAL in particular is a strong biochemical indicator of stress perception and activation of systemic acquired resistance (SAR). PAL serves as a critical regulatory point in the biosynthesis of salicylic acid (SA), a phytohormone central to long-distance signaling in plant defense, particularly against biotrophic pathogens such as *Hemileia vastatrix* (Abdelrhim et al., 2021; Salazar-Navarro et al., 2024). The observed induction of PAL by 12 mM SiNPs suggests that high doses can strongly activate this pathway; however, the potential phytotoxicity associated with higher concentrations, as indicated by photoinhibition and increased electrolyte leakage, may compromise overall plant health.

Interestingly, a subtle but detectable increase in PAL activity was also recorded at 1.5 and 3 mM, without signs of physiological stress. This suggests that low SiNP concentrations may prime the plant's metabolic machinery for a faster or stronger defense response upon pathogen challenge, a hallmark of induced resistance. Previous studies have reported similar PAL induction patterns in crops like rice and wheat after silicon supplementation, with corresponding enhancements in lignification and pathogen resistance (Du et al., 2022; Goswami et al., 2022).

Together, the activation of PAL and other defense-related enzymes supports the role of SiNPs as effective elicitors of immune signaling in *C. arabica*. The data further justify the selection of 1.5 mM SiNPs for infection assays using foliar disks, as this concentration maintains physiological homeostasis while priming key components of the plant's defense network.

3.5. Antioxidant Enzyme Activity

Peroxidase and catalase activities also increased with SiNP concentration, peaking at 12 and 6 mM, respectively. However, catalase activity at 1.5 mM was comparable to that of the control, and peroxidase remained within physiological limits, suggesting that these lower concentrations do not trigger oxidative stress but may instead sustain redox homeostasis. This enzymatic behavior further supports the notion that 1.5 mM SiNPs can act as elicitors without overwhelming the plant's antioxidant systems. Together, the moderate activation of antioxidant and defense-related enzymes at low doses provides biochemical evidence of a primed state, making these concentrations ideal for studying early plant–pathogen interactions under controlled conditions.

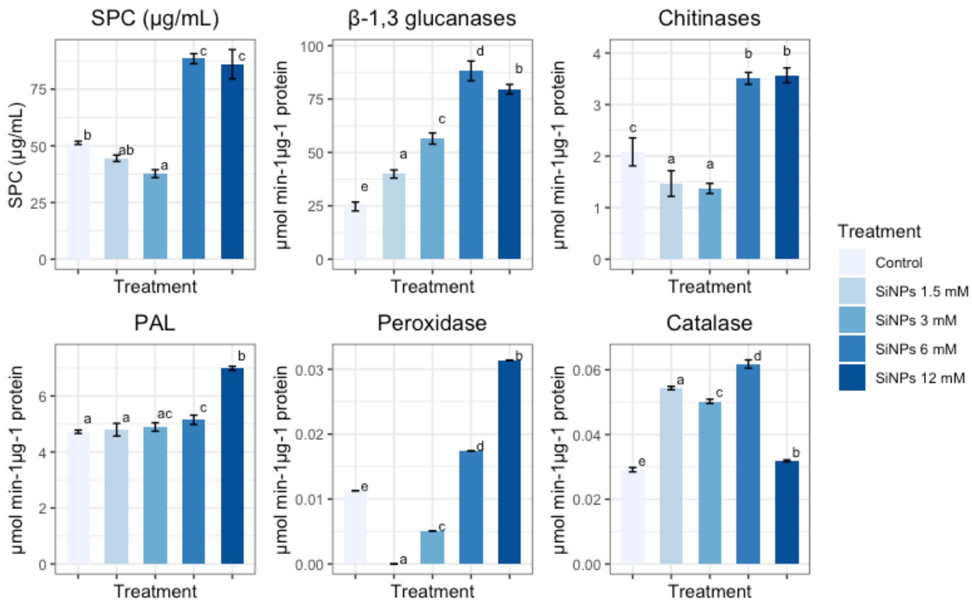


Figure 4. Biochemical responses of *Coffea arabica* var. *Bourbon* to foliar application of silica nanoparticles (SiNPs) under greenhouse conditions. Panels show soluble protein content (SPC) and specific activities of β-1,3-glucanases, chitinases, phenylalanine ammonia-lyase (PAL), peroxidase, and catalase. Different letters indicate statistically significant differences between treatments (Tukey's HSD, α = 0.05).

3.6. Electrolyte Leakage and Membrane Stability

Table 2 summarizes the effects of SiNP foliar application on electrolyte leakage (EL) and membrane stability index (MSI) in *Coffea arabica* var. *Bourbon* after 72 h. EL is widely recognized as a sensitive physiological indicator of membrane injury, reflecting increased ion efflux resulting from lipid peroxidation, oxidative stress, or structural disruption of the plasma membrane (Demidchik et al., 2014; Kafi et al., 2021; Nazim et al., 2024). Conversely, high MSI values indicate preserved membrane integrity and cellular homeostasis under stress conditions.

Plants treated with 1.5 mM SiNPs showed a significant reduction in EL and a concomitant increase in MSI compared to the untreated control. This outcome strongly suggests a protective effect on cellular membranes, likely mediated by enhanced antioxidant capacity, specifically through the activation of catalase and peroxidase enzymes (Figure 4). These enzymes reduce intracellular levels of reactive oxygen species (ROS), mitigating membrane lipid peroxidation and improving cellular resilience (Goswami et al., 2022). The data also align with earlier photochemical assessments (Section 3.2), where 1.5 mM SiNPs maintained optimal Fv/Fm and low NPQ, confirming the absence of stress-related photoinhibition at this concentration.

In contrast, intermediate SiNP concentrations (3 and 6 mM) resulted in elevated EL values and reduced MSI, indicating stress-induced membrane destabilization. This biphasic response is consistent with findings in other crops, where supra-optimal silicon levels can induce osmotic imbalance, interfere with ion homeostasis, or even disrupt membrane potential via increased K^+ efflux (Muhammad et al., 2022; Siddiqui et al., 2020). The oxidative stress associated with these concentrations may overwhelm the plant's antioxidant defense system, particularly at early stages of exposure.

Interestingly, the 12 mM treatment showed partial recovery in MSI, accompanied by lower EL compared to the 3 and 6 mM treatments. This apparent compensation may be attributed to the high peroxidase activity observed at this concentration (Figure 4), which likely contributes to late-stage ROS detoxification. However, this recovery was not paralleled by improvements in photochemical parameters (Section 3.2), suggesting that while membrane damage may be mitigated, other physiological systems remain compromised.

Taken together, these results support a dose-dependent, hormetic response to SiNPs, where low concentrations (1.5 mM) confer cytoprotective effects, while intermediate concentrations transiently disrupt membrane integrity, potentially leading to recovery at high doses. This highlights the importance of fine-tuning nanoparticle concentration in field applications to avoid unintended phytotoxic effects while maximizing stress tolerance.

Table 2. Electrolyte leakage (EL) and membrane stability (MS) in *Coffea arabica* var. *Bourbon* 72 h after foliar application of silica nanoparticles (SiNPs) at different concentrations.

Variable	Control	SiNPs 1.5 mM	SiNPs 3 mM	SiNPs 6 mM	SiNPs 12 mM
EL	30.99 ± 6.16^{ab}	17.99 ± 2.39^{c}	37.74 ± 2.42^{a}	33.715 ± 0.307^{a}	24.698 ± 0.78^{bc}
MS	69.01 ± 6.16^{bc}	82.01 ± 2.39^{a}	62.26 ± 2.42^{c}	66.285 ± 0.307^{c}	75.302 ± 0.78^{ab}

Data are mean ± standard deviation (n = 4). EL = electrolyte leakage (%); MS = membrane stability (%). Different letters indicate statistically significant differences according to Tukey's HSD test (α = 0.05).

3.7. Microscopic Staining and Membrane Integrity Assays

To further investigate the potential role of SiNPs as defense elicitors in *Coffea arabica*, a complementary histological and physiological evaluation was performed using leaf disks subjected to *H. vastatrix* inoculation. Disks were treated with 1.5 mM SiNPs or sterile distilled water (control), a concentration selected based on its demonstrated elicitor activity in enzymatic assays, low phototoxicity, and stable photochemical performance (see Sections 3.2–3.5).

3.7.1. Trypan Blue and Lactophenol Blue Staining

Trypan blue and lactophenol blue stains were selected to monitor the viability of *H. vastatrix* urediniospores and the development of fungal infection structures on the abaxial surface of *Coffea arabica* leaf disks. Leaf imprints and staining were performed at 24 and 48 hours post-inoculation. Lactophenol blue-stained imprints (Figure 5) revealed that, at 24 hours, both treatments showed comparable behavior with no visible germ tube formation. By 48 hours, control samples exhibited clear signs of early infection events, including germ tube emergence, stomatal penetration, and appressorium formation. In contrast, urediniospores on SiNP-treated disks remained ungerminated.

Trypan blue staining (Figure 6) showed a similar pattern. At 24 hpi, urediniospores from both treatments appeared viable, as indicated by their uniform staining. However, at 48 hpi, urediniospores from the SiNP-treated group exhibited intense blue staining, suggesting loss of membrane integrity and reduced viability. These findings suggest that SiNP treatment may trigger plant-mediated defense responses capable of suppressing fungal development during the early infection stages.

This mode of action aligns with the characteristics of the SiNPs used in this study. The Sol-Gel synthesis using sodium metasilicate in aqueous conditions is known to yield amorphous, spherical nanoparticles with low intrinsic antifungal activity and limited ROS generation (Liang et al., 2023; Owoeye et al., 2021b). While silicic acid release was not directly quantified in this study, previous research has shown that amorphous SiNPs synthesized via similar methods can gradually release bioavailable silicon. Furthermore, SiNPs with abundant surface silanol groups may be absorbed and translocated by plants through the same pathways as molecular silicic acid (Bhat et al., 2021; Yan et al., 2024). This soluble silicon has been associated with the activation of salicylic acid (SA)-dependent signaling pathways and the induction of systemic acquired resistance (SAR) in various crops (Fauteux et al., 2005; Ranjan et al., 2021). Therefore, the observed inhibition of *H. vastatrix* germination and reduction in spore viability on SiNP-treated tissues is likely the result of an indirect, host-mediated defense response, supporting the potential use of these nanoparticles as priming agents in integrated coffee disease management strategies.

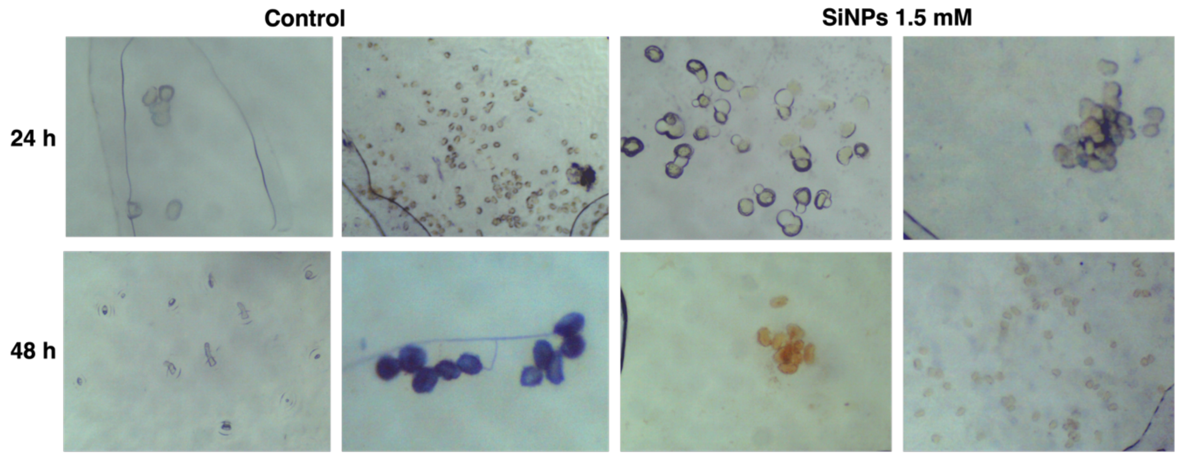


Figure 5. Development of *Hemileia vastatrix* urediniospores on the abaxial surface of *Coffea arabica* var. *Bourbon* leaf disks stained with lactophenol blue at 24 and 48 hours post-inoculation *(hpi).* Left panels: Control treatment. Right panels: SiNP-treated disks (1.5 mM). At 24 hpi, both treatments showed similar patterns with dispersed, non-germinated urediniospores. By 48 hpi, control samples exhibited visible signs of early infection, including germ tube emergence, penetration through stomata, and formation of appressoria. In contrast, urediniospores on SiNP-treated disks remained ungerminated and showed no signs of infection structure development, suggesting inhibition of fungal progression.

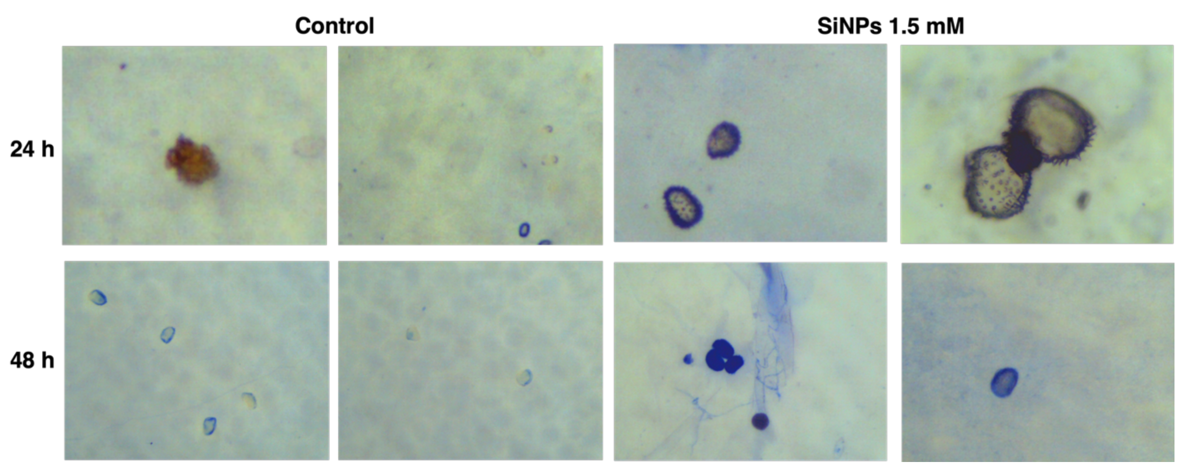


Figure 6. Viability of *Hemileia vastatrix* urediniospores on the abaxial surface of *Coffea arabica* var. *Bourbon* leaf disks stained with trypan blue at 24 and 48 hours post-inoculation (hpi). Left panels: control treatment; right panels: SiNP-treated disks (1.5 mM). At 24 hpi, urediniospores in both treatments stained uniformly, indicating

maintained membrane integrity. At 48 hpi, urediniospores on SiNP-treated disks exhibited intense blue staining, consistent with membrane disruption and viability loss.

3.7.2. Electrolyte Leakage and Membrane Stability

Although chlorophyll fluorescence and SPAD measurements are widely used to monitor plant responses to stress, these assessments were omitted from the infection assay to avoid cross-contamination of fluorometer clips and chlorophyll meters with *H. vastatrix* urediniospores. Instead, electrolyte leakage (EL) and the membrane stability index (MSI) were employed as robust indicators of membrane integrity and early physiological changes associated with biotic stress.

As shown in Figure 7, the physiological status of leaf disks was evaluated under three conditions: CN (non-inoculated control), CI (inoculated control), and SiNPsI (SiNP-treated and inoculated). At 24 hours post-inoculation, SiNPsI-treated disks exhibited a significant increase in EL compared to CN and CI, suggesting an initial membrane destabilization. This early response may reflect the transient stress associated with SiNP recognition and uptake, as well as the onset of defense signaling cascades triggered by the nanomaterial.

However, at 48 and 72 hours, SiNPsI-treated samples showed a marked reduction in EL and a corresponding increase in MSI, indicating a recovery of membrane integrity and physiological homeostasis. This biphasic response is consistent with a priming effect, wherein plants transiently activate defense-related signaling pathways without undergoing sustained damage, ultimately enhancing their resilience to pathogen attack.

The improved membrane stability in SiNP-treated tissues corresponds well with the histological observations (Figures 5 and 6), where urediniospore germination was arrested and early signs of spore death were evident. Together, these findings support the hypothesis that SiNPs act as elicitors of plant defense, potentially by modulating membrane-associated signaling and activating antioxidant and structural responses.

The concentration used (1.5 mM) was selected based on previous results demonstrating minimal phytotoxicity, low ROS-associated damage, and reduced NPQ values in whole-plant assays. These findings highlight its suitability for use in pathoassays involving sensitive tissue systems such as leaf disks. Moreover, the EL/MSI results reinforce the potential of low-dose SiNPs to confer membrane-level protection during early pathogen colonization stages.

Taken together, the results from membrane integrity assays (EL/MSI) and histochemical stains (Figures 5–7) align with the biochemical evidence obtained from whole-plant enzymatic evaluations. The increase in defense-related enzyme activity—including PAL, chitinases, and β-1,3-glucanases—particularly at low to moderate SiNP concentrations, suggests that foliar application of SiNPs can prime coffee plants for enhanced defense readiness. In the leaf disk infection model, this primed state was reflected in the suppression of *H. vastatrix* germination structures and improved membrane stability over time. The activity of PAL is especially relevant, as it initiates the phenylpropanoid pathway leading to lignin and phenolic compound biosynthesis—critical components in cell wall reinforcement and pathogen restriction (Goswami et al., 2022; Saberi Riseh et al., 2023). These coordinated responses across molecular, physiological, and structural levels support the hypothesis that SiNPs synthesized via the Sol-Gel method act as effective, non-toxic elicitors capable of activating systemic acquired resistance (SAR) in *C. arabica*.

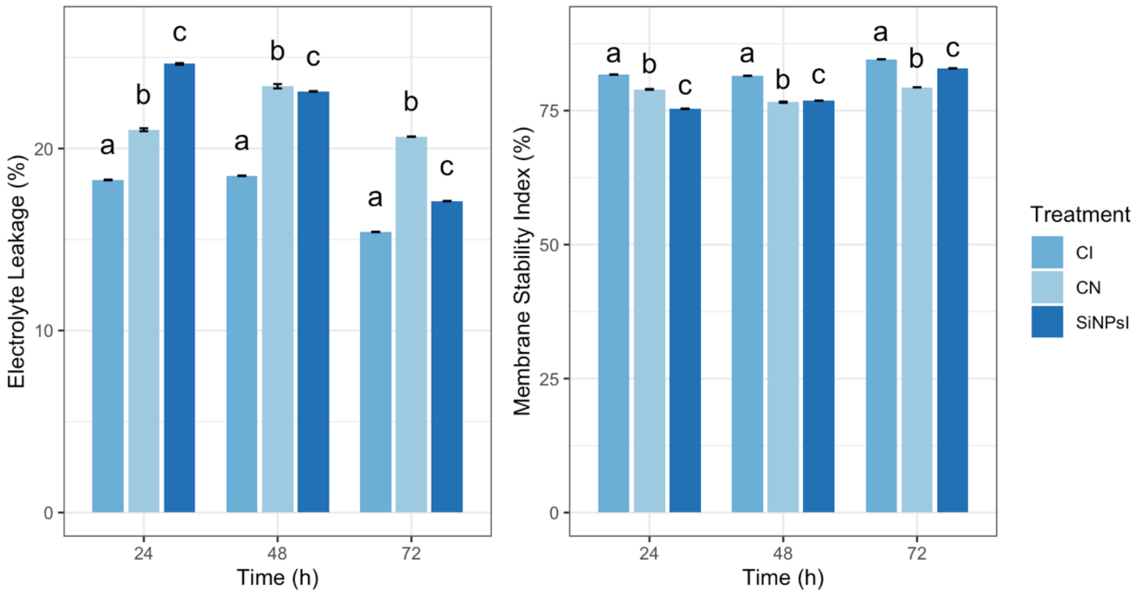


Figure 7. Electrolyte leakage (EL, left) and membrane stability index (MSI, right) in *Coffea arabica* var. *Bourbon* leaf disks subjected to three treatments: CN (non-inoculated control), CI (inoculated control), and SiNPsI (1.5 mM SiNP-treated and inoculated). Measurements were taken at 24, 48, and 72 hours post-inoculation (hpi). At 24 hpi, the SiNPsI treatment showed elevated EL, indicating initial membrane perturbation likely related to the plant's recognition of the nanomaterial and defense activation. By 72 hpi, EL decreased and MSI improved in SiNP-treated disks, suggesting recovery of membrane integrity and possible adaptation to the stressor. Different letters indicate significant differences among treatments at each time point (Tukey's test, $p < 0.05$).

4. Conclusions

This study demonstrates that foliar application of silica nanoparticles (SiNPs), synthesized via the Sol-Gel method, can effectively modulate physiological and biochemical responses in *Coffea arabica* var. *Bourbon* under greenhouse conditions. At low to moderate concentrations (1.5–3 mM), SiNPs preserved photosynthetic efficiency, minimized photoinhibition (as reflected in stable Fv/Fm values), and reduced non-photochemical quenching (NPQ), indicating low phytotoxicity. Notably, 1.5 mM SiNPs also enhanced membrane stability and reduced electrolyte leakage, suggesting improved cell integrity.

Higher concentrations (6–12 mM) induced the activity of key antioxidant and pathogenesis-related enzymes, including β-1,3-glucanases, chitinases, phenylalanine ammonia-lyase (PAL), peroxidase, and catalase. These results point to the activation of salicylic acid–associated defense pathways. In parallel, histochemical analyses using trypan blue and lactophenol blue revealed that 1.5 mM SiNPs suppressed the germination and viability of urediniospores in inoculated leaf disks, reinforcing the role of SiNPs as defense elicitors rather than direct antifungal agents. This was further supported by physiological assays in the infection model, where electrolyte leakage decreased and membrane stability recovered over time in SiNP-treated disks.

Together, these findings provide compelling evidence that SiNPs can prime coffee plants for enhanced defense readiness against *Hemileia vastatrix* without compromising plant health. The application of amorphous, degradable SiNPs may represent a sustainable and non-toxic strategy to bolster crop resilience. Future research should focus on field validation, long-term impacts, and the use of functionalized nanoparticles tailored to specific plant-pathogen interactions for integrated disease management in coffee production systems.

5. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

6. Author Contributions

SA: Writing – original draft. RV: Writing – review & editing. JJ: Writing – review & editing. VB: Writing – review & editing DD: Writing – review & editing TO: Writing – review & editing BU: Writing – review & editing. GD: Writing – review & editing.

7. Acknowledgments

The author Salazar-Navarro (CVU 968662) acknowledges the Secretaria de Ciencia, Humanidades, Tecnologías e Innovación (SECIHTI) for the support offered during his postgraduate studies in the Doctorado en Ciencias Agrícolas at the Instituto de Ciencias Agrícolas in the Universidad Autónoma de Baja California.

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CAPÍTULO 7. Estimulación de defensas en *Coffea arabica* mediante nanopartículas de sílice funcionalizadas con COS y Cu^{2+}

Título original del manuscrito: Enhanced Defense Priming in *Coffea arabica* var. Borbon Using Silica Nanoparticles Functionalized with Chitosan Oligosaccharides and Copper Ions

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Este capítulo corresponde a un manuscrito en preparación. Actualmente está considerado para su envío a la revista **Plant Nano Biology**, *en el área de elicitación vegetal y nanotecnología funcional.*

Resumen

Este estudio evaluó el potencial de nanopartículas de sílice funcionalizadas con oligómeros de quitosano (SiNPs-COS) y iones cúpricos (SiNPs-COSCu) como elicitores moleculares y bioquímicos en *Coffea arabica* var. *Bourbon*. Las SiNPs se sintetizaron por el método Sol-Gel y fueron caracterizadas por FTIR, DLS, SEM y EDS. Se aplicaron foliarmente y, tras 72 h, se analizó la expresión de genes asociados a defensa (*PAL, PR1, PR5, CaNDR1, NBS-LRR, β-1,3-glucanasa*) junto con parámetros bioquímicos (actividad de PAL, quitinasa, glucanasa, peroxidasa, catalasa), así como integridad de membrana mediante fugas electrolíticas e índice de estabilidad. Las formulaciones funcionalizadas mostraron efectos específicos en la activación de SAR, señalización por ROS, y balance antioxidante, con inducciones de hasta 314 veces en *β-1,3-glucanasa* y 163 veces en *CaNDR1*, dependiendo del tratamiento. Los resultados confirman que las SiNPs funcionalizadas pueden actuar como nanoelicitores selectivos capaces de inducir defensas tempranas en plantas de café bajo condiciones no infecciosas.

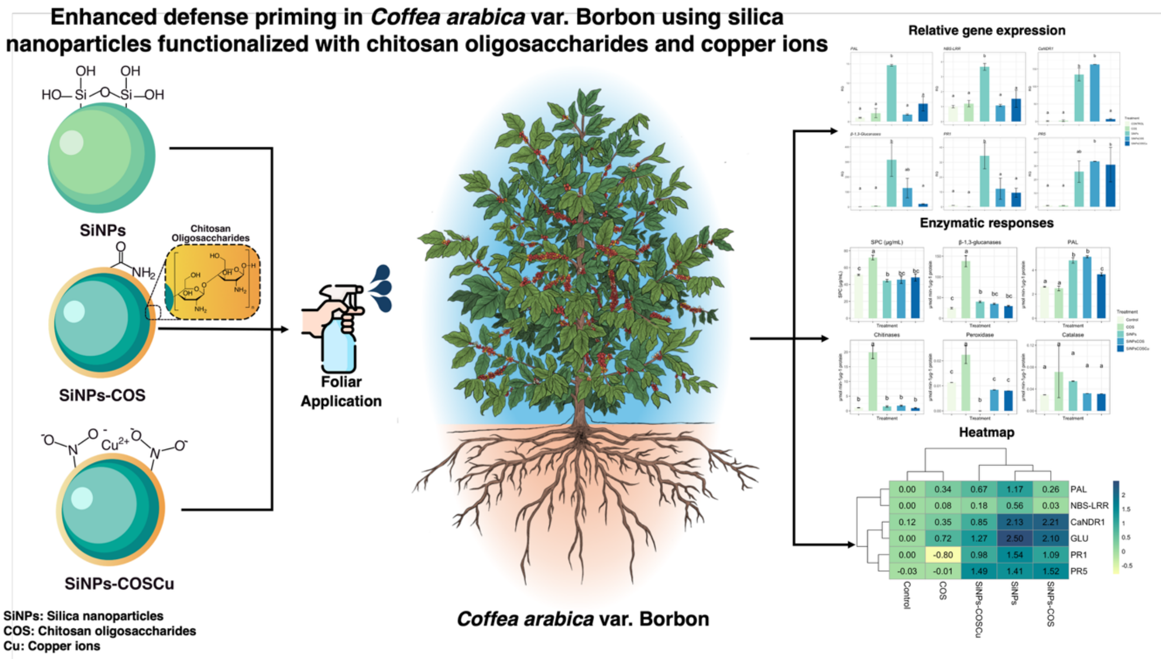
Palabras clave: inmunidad vegetal, resistencia adquirida sistémica, nanobiotecnología, elicitación foliar, nanopartículas de sílice, *Coffea arabica*

Abstract

Coffee (*Coffea arabica*) is highly vulnerable to biotic stressors that compromise plant health and productivity. This study evaluated the use of silica nanoparticles (SiNPs) functionalized with chitosan oligosaccharides (COS) and copper ions (Cu^{2+}) as foliar elicitors of molecular and biochemical defense responses in *C. arabica* var. *Bourbon*. SiNPs were synthesized via the sol–gel method and characterized by FTIR, DLS, and SEM-EDX. Treatments were applied by foliar spray, and samples were analyzed 72 hours later. The formulations modulated the expression of key defense-related genes (*PAL, PR1, PR5, CaNDR1, NBS-LRR*, and *β-1,3-glucanase*), with *β-1,3-glucanase* increasing up to 314-fold in SiNPs-treated plants and *CaNDR1* up to 163-fold in response to SiNPs-COS. SiNPs-COSCu induced strong expression of *PR1* and *PR5* (34- and 31-fold, respectively). Enzymatic responses showed treatment-specific patterns. PAL activity was highest in SiNPs (4.80 ± 0.23) and SiNPs-COS (5.10 ± 0.08 $\mu mol\ min^{-1}\ \mu g^{-1}$ protein). COS-treated plants exhibited elevated β-1,3-glucanase (137.94 ± 13.36), chitinase (19.90 ± 2.17), and peroxidase (0.02 ± 0.00) activity. Catalase activity was notably elevated in SiNPs-COSCu-treated plants (0.03 ± 0.00), suggesting a ROS-regulating effect linked to copper ions. Physiological responses confirmed membrane protection under SiNPs (EL: 17.99 ± 2.39%; MS: 82.01 ± 2.39%) and SiNPs-COS (EL: 12.95 ± 0.06%; MS: 87.05 ± 0.06%) treatments, while SiNPs-COSCu showed higher EL and reduced MS, indicating oxidative imbalance. These findings highlight the potential of functionalized SiNPs as preventive nanoelicitors capable of enhancing early defense responses in coffee under non-infectious conditions.

Keywords: Plant immunity, Nanobiotechnology, Systemic acquired resistance, Foliar elicitor, Sol-Gel

Graphical abstract



1. Introduction

Coffee production represents a key agricultural sector in Mexico, holding substantial socioeconomic importance due to its significant role in national trade and export markets. Approximately 80% of national coffee production is exported, while the remaining 20% supports domestic consumption (Gabriel-Hernández and Barradas, 2024). Mexico consistently ranks among the top 10–12 global coffee producers (Calvillo-Arriola and Sotelo-Navarro, 2024), with Chiapas, Veracruz, Puebla, and Oaxaca being the main producing states (Gaona Ulaje et al., 2024). The two main cultivated species are *Coffea arabica* and *Coffea canephora* (commonly known as Arabica and Robusta coffee, respectively), with *C. arabica* accounting for approximately 96% of total cultivation (Salazar-Navarro et al., 2024).

Coffee crops face increasing challenges due to both abiotic and biotic stressors. Among the most critical threats are climate change, nutrient deficiencies, pests, and phytopathogens (García-Méndez et al., 2024). Rising global temperatures are particularly concerning as they reduce the availability of suitable agroclimatic zones for coffee cultivation and alter pest and pathogen dynamics. For instance, elevated temperatures have been associated with increased aggressiveness of the coffee berry borer (*Hypothenemus hampei*) and coffee leaf rust (CLR), caused by *Hemileia vastatrix* (Ayalew et al., 2024; Paragon Ritonga and Kwon, 2024). In response, producers often increase shade coverage to mitigate heat stress—altering microclimates in ways that can inadvertently promote pathogen proliferation (Ayalew et al., 2024; García-Méndez et al., 2024; Paragon Ritonga and Kwon, 2024).

Conventional management of CLR includes the application of copper-based fungicides and the use of resistant cultivars. However, these strategies face important limitations. The protective effect of copper compounds is short-lived, as Cu^{2+} ions are easily washed away by rainfall due to their solubility, reducing their efficacy in tropical climates (Granados and Zambolim, 2019). Meanwhile, the durability of genetic resistance is undermined by the high variability and adaptability of *H. vastatrix* races, which can overcome host resistance in relatively short periods (Julca-Otiniano et al., 2024). Additionally, the repeated use of synthetic fungicides raises environmental concerns and may lead to soil and plant toxicity (Gao et al., 2024).

In light of these challenges, there is growing interest in the use of elicitors—compounds that stimulate plant defense mechanisms—as a sustainable alternative to conventional disease control. Among these, natural biopolymers such as chitosan and its oligosaccharide derivatives (COS), as well as nanomaterials like silica nanoparticles (SiNPs), have shown promise in inducing immune responses in plants. COS are recognized as pathogen-associated molecular patterns (PAMPs) and can trigger defense-related gene expression through salicylic acid (SA)-mediated signaling (Gao et al., 2024; Shinde et al., 2024). SiNPs can act as elicitors by modulating reactive oxygen species (ROS) and activating basal defense pathways (Deng et al., 2024; Masood et al., 2024).

Furthermore, functionalization of SiNPs with biopolymers or metal ions such as Cu^{2+} has been proposed as a strategy to improve the stability, bioavailability, and functional properties of these nanomaterials. Such biofunctionalized nanoparticles can enhance plant uptake, prolong the release of active molecules, and selectively modulate key components of the plant immune system (Sarkar et al., 2022). Despite promising results in various crops, few studies have explored the use of Si-based nanocomposites as elicitors in coffee plants or their effects at the molecular level.

This study aims to evaluate the physicochemical properties and biological effects of SiNPs functionalized with COS and Cu^{2+} (SiNPs-COS and SiNPs-COSCu) in *Coffea arabica* var. *Bourbon*. Specifically, we assess their impact on the expression of defense-related genes, enzymatic responses, and cell membrane stability as indicators of immune activation. Our findings provide insights into the design of targeted nanomaterials for sustainable disease management in perennial crops.

2. Materials and methods

2.1. Synthesis of Silica Nanoparticles (SiNPs)

SiNPs were synthesized from 0.3 M sodium metasilicate (PQ Corporation, USA) following Sol-Gel principles, according to our previously described methods (Salazar-Navarro et al., 2023; Salazar-Navarro and Salas-Valdez, 2022). The sodium metasilicate solution was treated with a cation exchange resin (Bulk Reef Supply®, Sigma-Aldrich) to reduce Na^+ ion concentration and decrease the pH from 14 to 10. To initiate the Sol-Gel reaction, 5 mL of ethanol (Fermont®, Mexico) was added dropwise under constant stirring at 300 rpm for 15 min. The mixture was then homogenized at 2,000 rpm for 30 min using an industrial homogenizer (ROSS®, model HSM100LSK1, USA). Silwet L-77 (Momentive, USA) was added at 1% v/v in distilled water (100 mL total volume). The resulting suspension was refluxed at 105 °C for 30 min.

2.2. Synthesis of Chitosan Oligosaccharides (COS)

Chitosan oligosaccharides (COS) were synthesized from low molecular weight chitosan (Sigma-Aldrich®, USA) according to our previously reported method (Salazar-Navarro et al., 2025). The resulting COS had an average molecular weight of 3,549.9 ± 0.33 Da and a degree of deacetylation (DD) of 76.64 ± 1.12%. The hydrolysis process was carried out as previously described (Salazar-Navarro et al., 2023), using a combination of acid treatment with 1% glacial acetic acid, oxidative hydrolysis with 2% hydrogen peroxide, and mechanical hydrolysis via microwave irradiation at 700 W for 1 minute.

2.3. Functionalization of SiNPs with COS (SiNPs-COS) and Cu^{2+} ions (SiNPs-COSCu)

To functionalize the SiNPs, the synthesized nanoparticles were suspended in distilled water under constant stirring at 300 rpm, reaching a final concentration of 1.5 mM. Chitosan oligosaccharides (COS) were then added dropwise to the suspension to obtain a final concentration of 0.5% (v/v). Surface modification was promoted by electrostatic interactions between negatively charged SiNPs and the cationic COS chains, following our previously reported method (Salazar-Navarro et al., 2023). The resulting SiNPs-COS suspension was stirred for 15 min at 300 rpm. Subsequently, $CuSO_4$ solution (0.1 M) was added dropwise to reach a final copper ion concentration of 5 mM. The mixture was stirred for an additional 15 min at 300 rpm to allow Cu^{2+} adsorption, yielding the final SiNPs-COSCu formulation.

2.4. SiNPs, SiNPs-COS, and SiNPs-COSCu characterization

The structural and chemical composition of SiNPs, SiNPs-COS, and SiNPs-COSCu was analyzed using Fourier-transform infrared spectroscopy (FTIR, Agilent®, USA), which allowed the identification of functional groups associated with COS and Cu^{2+} on the SiNP surface.

Dynamic light scattering (DLS) and zeta potential (ζ-potential) measurements were performed using a Litesizer 500 (Anton Paar®, Austria) to determine the hydrodynamic diameter, polydispersity index (PDI), and colloidal surface charge. Measurements were conducted at 25 °C in distilled water, with three independent readings per sample. These parameters were used to evaluate changes in particle size distribution, dispersion stability, and electrostatic behavior following functionalization.

All analyses were carried out on freshly prepared nanoparticle suspensions to minimize agglomeration artifacts. Comparative data across treatments were used to confirm successful functionalization and assess the impact of COS and Cu^{2+} incorporation on nanoparticle properties.

Additionally, morphological characterization of dried samples was performed using scanning electron microscopy (SEM, JEOL®, model JSM-6010LA, Japan), which provided information on particle shape, aggregation patterns, and surface texture. Elemental analysis was carried out using energy-dispersive X-ray spectroscopy (EDX) coupled to SEM to confirm the presence of functionalizing elements. The characteristic signals of silicon (Si), oxygen (O), nitrogen (N), and copper (Cu) were used as indicators of COS and Cu^{2+} incorporation onto the silica matrix.

2.5. Plant material and treatments

Coffea arabica var. *Bourbon* plants, six months old, were grown under greenhouse conditions in polyethylene bags (6.7 × 6 × 26 cm) filled with a peat moss:agrolite substrate (3:1 v/v). Plants were irrigated twice weekly with 100 mL of potable water. Foliar treatments were applied by spraying 2 mL of each formulation onto the adaxial surface of the upper leaves using a handheld atomizer. The evaluated treatments included distilled water, as control, unmodified silica nanoparticles (SiNPs, 1.5 mM), silica nanoparticles functionalized with chitosan oligosaccharides (SiNPs-COS), and silica nanoparticles functionalized with chitosan oligosaccharides and Cu^{2+} ions (SiNPs-COSCu). Treated plants were maintained under greenhouse conditions for 72 hours before tissue sampling for biochemical and molecular analyses. Each treatment was conducted with four biological replicates.

2.6. Molecular response of *C. arabica* var. *Bourbon* plants

A total of 50 mg of the third fully expanded leaf from each treatment was collected and ground in liquid nitrogen. Total RNA was extracted using the Plant/Fungi Total RNA Purification Kit (NORGEN®, Cat. No. 25800), following the manufacturer's instructions. RNA quantity and integrity were assessed by spectrophotometric analysis using a Nanodrop One® (Thermo Fisher Scientific, USA), and by electrophoresis on 2% agarose gels at 80 V and 400 mA for 40 min.

First-strand cDNA synthesis was performed using 1 µg of total RNA and the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. No. K1622). Reverse transcription quantitative PCR (RT-qPCR) was carried out using the Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Cat. No. K0221) in a StepOne® Real-Time PCR System (Applied Biosystems®, USA) under standard cycling conditions.

A panel of six defense-related genes was selected: *PAL*, *CaPR1*, *CaPR5*, *CaNBS-LRR*, *CaNDR1b*, and *C. arabica β-1,3-glucanase* (*GLU*). The reference gene *CaUbiE2* was used for normalization (Table 1). Primers were designed based on *C. arabica* gene sequences available in the NCBI database, using Primer3Plus (www.primer3plus.com). All primers were synthesized by T4-Oligo (www.t4oligo.com). Primer sequences and references are listed in Table 1.

Relative gene expression was calculated using the ΔΔCt method. All reactions were performed in technical replicates, and the relative quantification (RQ) values were expressed as fold changes compared to the untreated control group.

Table 1. RT-qPCR primer sequences used for gene expression analysis.

Forward (F) and reverse (R) primer sequences used to amplify defense-related genes in *Coffea arabica*, including *PAL*, *CaPR1*, *CaPR5*, *CaNBS-LRR*, *CaNDR1b*, and *C. arabica β-1,3-glucanase*. Primer sequences were obtained from previous reports as indicated.

Gene	Primer sequence (5'-3')	Ref
C. arabica B-1,3-glucanase	F: CTTCTTTGATGGGGCTGCTAA R: ATATAACCAGGCCGAAGTGGG	(Guzzo et al., 2009)
CaNDR1b	F: CTTACAGGGCGGTGTCAAAT R: TACCACTAGCCCAGGACAGC	(Couttolenc-Brenis et al., 2021)
CaUbiE2	F: CCATTTAAACCCCCAAAGGT R: GGTCCAGCTTCGAGCAGTAG	(Couttolenc-Brenis et al., 2021)
CaNBS-LRR	F: CCAAAAACTTTGGGTTGGTG R: TCCATTGCATTCTCATCTG	(Couttolenc-Brenis et al., 2021)
CaPR1	F: CAGGAATGCGGGCATTATAC R: CAATCGCATGGGTTTGATAA	(Couttolenc-Brenis et al., 2021)
CaPR5	F: CTGCCTGAGTTGCAGCAATA R: TTTCCCTTGTTGATGGCTTC	(Couttolenc-Brenis et al., 2021)

Gene	Primer sequence (5’-3’)	Ref
C. arabica B-1,3-glucanase	F: CTTCTTTGATGGGGCTGCTAA R: ATATAACCAGGCCGAAGTGGG	(Guzzo et al., 2009)
PAL	F: CATCAGGGCTTCGACAAAAT R: CGGGTGTTATCCATCGAGAC	(Couttolenc-Brenis et al., 2021)

2.7. Specific enzymatic activity response of *C. arabica* var. *Bourbon* plants

Enzymatic activity assays were performed on the same plant material and treatments described in Section 2.5, following previously reported methodologies (Luján-Hidalgo et al., 2020; Salazar-Navarro et al., 2025). Specifically, leaf samples were collected 72 hours after foliar application of distilled water (control), SiNPs, SiNPs-COS, or SiNPs-COSCu, under greenhouse conditions. A total of 50 mg of tissue from the second fully expanded leaf was collected per plant and ground in liquid nitrogen. The tissue was homogenized in extraction buffer containing 0.1 M Tris-HCl (pH 8.0), 10% glycerol, 1% polyvinylpyrrolidone (PVP), 0.1% ascorbic acid, and 5% β-mercaptoethanol. Extracts were centrifuged at 15,000×g for 15 min at 4 °C, and the supernatants were stored at −4 °C until analysis.

Enzymatic activities were determined spectrophotometrically using a Nanodrop One® (Thermo Fisher Scientific, USA) following the methods described by (Castro et al., 2011; Luján-Hidalgo et al., 2020; Reichel et al., 2022; Salazar-Navarro et al., 2025; Santos-Espinoza et al., 2021), with slight modifications:

- **PAL activity** was measured by incubating 5 µL of protein extract with 145 µL of 50 mM Tris-HCl (pH 8.8) and 50 µL of 50 mM L-phenylalanine at 37 °C for 20 min. Absorbance was recorded at 280 nm (Reichel et al., 2022).
- **β-1,3-glucanase activity** was determined by hydrolysis of 0.15% laminarin in 50 mM sodium acetate buffer (pH 5), followed by DNS colorimetric quantification of reducing sugars at 515 nm (Luján-Hidalgo et al., 2020).

- **Chitinase activity** was assayed by incubating 0.05% colloidal chitin in sodium acetate buffer (pH 5), and measuring N-acetylglucosamine release at 420 nm after addition of potassium ferrocyanide and heating (Castro et al., 2011).
- **POD activity** was evaluated by monitoring guaiacol oxidation in the presence of 10 mM H_2O_2 at 470 nm (Santos-Espinoza et al., 2021).
- **CAT activity** was assessed by following the decomposition of H_2O_2 at 240 nm in Tris-HCl buffer (pH 8) with 5 mM EDTA (Santos-Espinoza et al., 2021).

Total soluble protein content (SPC) was quantified by the Bradford method, and enzymatic activity was expressed as specific activity (units per mg of protein). All assays were conducted in technical replicates using four biological replicates per treatment.

2.8. Statistical analysis

All experiments were performed with four biological replicates and three technical repetitions per treatment. The results were expressed as mean ± standard deviation (SD). Statistical analyses, data visualization, and clustering were conducted using RStudio v2025.05.0+496 (R Foundation for Statistical Computing, Austria) using base R functions and the pheatmap, ggplot2, and tidyverse packages. One-way analysis of variance (ANOVA) followed by Tukey's test was used to determine statistically significant differences between treatments ($p < 0.05$). Gene expression data were log-transformed prior to clustering analysis. A heatmap was constructed using hierarchical clustering with Euclidean distance to visualize expression patterns among treatments.

3. Results and discussion

3.1. SiNPs, SiNPs-COS, and SiNPs-COSCu characterization

Figure 1 presents the SEM micrographs and EDS analysis of the synthesized SiNPs and their functionalized counterpart (SiNPs-COSCu). The SEM image of SiNPs (Figure 1A) reveals a predominantly spherical morphology, consistent with our previously reported method for synthesizing silica nanoparticles from sodium metasilicate, based on pH control and electrolyte concentration (Salazar-Navarro and Salas-Valdez, 2022). The corresponding EDS spectrum confirms a chemical composition dominated by carbon (48.59%), oxygen (42.62%), and silicon (6.83%), with minor traces of aluminum (1.96%).

In contrast, the SEM image of SiNPs-COSCu (Figure 1B) shows an aggregated globular morphology, indicative of surface modification. The EDS analysis revealed a composition enriched in carbon (35.37%), oxygen (25.03%), and copper (24.25%), along with minor signals of sulfur (8.50%), nitrogen (6.23%), and silicon (0.62%). The sulfur content corresponds to the $CuSO_4$ used as the copper source.

The increased presence of C, O, N, and Cu supports the successful deposition of COS and Cu^{2+} onto the nanoparticle surface (Hongfeng et al., 2021; Singh et al., 2024). The decrease in Si content may be attributed to the dilution effect during functionalization (from 0.3 M to 1.5 mM), along with the higher concentrations of COS (0.5% v/v) and Cu (5 mM).

Functionalization is likely driven by covalent and non-covalent interactions between silanol groups (Si–OH) on the nanoparticle surface and functional groups from COS, such as hydroxyl (–OH) and amino ($–NH_2$) groups. These interactions promote both hydrogen bonding and copper chelation, enhancing structural stability, dispersibility, and potential bioactivity of the hybrid material (Onoka and Hilonga, 2025; Saleh et al., 2022; Sportelli et al., 2017).

The globular aggregation observed in SiNPs-COSCu may be a consequence of these intermolecular interactions, further reinforced by electrostatic attraction between negatively charged SiNPs and the protonated amine groups of COS. The acidic conditions and sulfate ions from $CuSO_4$ may also facilitate Cu^{2+} retention and coordination (Guibal et al., 2014).

These findings correlate with the DLS results shown in Figures 2 and 3. The zeta potential (ζ, mV) results are presented in figure 2, where the surface modification of SiNPs is evident from their ζ-potential change. The ζ-potential is a commonly used parameter to confirm the surface modification or functionalization of colloids (Heidari et al., 2023). The ζ-potential shifts from -32.3 mV in SiNPs to 33.9 mV in SiNPs-COS and 28 mV in SiNPs-COSCu. This ζ-potential modification can be attributed to the charges present in chitosan and its oligomers (COS), with values of approximately 40 mV and 20.9 mV, respectively. The observed shift in ζ-potential polarity is a direct indicator of successful surface functionalization, confirming the modification of SiNPs into SiNPs-COS and SiNPs-COSCu. These results align with our previously reported methodology for tailoring the surface properties of SiNPs through functionalization and other research that successfully demonstrates SiNPs functionalization with chitosan and its derivatives (Dhinasekaran et al., 2020; Heidari et al., 2021; Salazar-Navarro et al., 2023).

The ζ-potential measurements underscore the enhanced colloidal stability achieved through the functionalization process. The shift from a highly negative ζ-potential in SiNPs to positive or near-neutral values in SiNPs-COS and SiNPs-COSCu indicates a significant reduction in interparticle repulsion. This change is likely driven by electrostatic interactions between the amine groups in COS and the hydroxyl-rich SiNP surface (Heidari et al., 2023). Furthermore, these findings suggest that the functionalization contributes to the stabilization of the nanoparticle suspensions, ensuring a stable dispersion for SiNPs, SiNPs-COS, and SiNPs-COSCu. Notably, ζ-potential values exceeding +30 mV or falling below -30 mV are generally indicative of sufficient electrostatic repulsion to maintain colloidal stability (Majid Abdouss, 2024; Muthukrishnan, 2015).

The DLS results for SiNPs, SiNPs-COS, and SiNPs-COSCu, presented in Figure 3 (graphs A, B, and C, respectively), illustrate a clear trend of increasing particle size with each functionalization step. The hydrodynamic diameter increases from 1.35 nm in SiNPs to 6.96 nm in SiNPs-COS and reaches 17.15 nm in SiNPs-COSCu. This progressive growth in size can be attributed to the successful deposition of COS and Cu layers onto the SiNPs surface during the functionalization process.

The significant increase in size after COS and Cu incorporation suggests not only effective surface coverage but also potential aggregation due to intermolecular interactions among functionalized nanoparticles. These results are consistent with the ζ-potential findings, where changes in surface charge further confirm the modification of the nanoparticles. This size increase also aligns with previous studies that report similar trends in functionalized nanoparticles (Arvejeh et al., 2025; Sanità et al., 2020).

To corroborate the functionalization, FTIR analyses were performed. The IR spectra of each precursor (chitosan and COS) and modified nanoparticles (SiNPs, SiNPs-COS, and SiNPs-COSCu) are shown in Figure 4. While chitosan and COS displayed similar spectral patterns, notable differences in transmittance intensity and peak position were observed, which can be attributed to variations in molecular weight and exposure of functional groups following hydrolysis.

Characteristic stretching vibrations resulting from N–H (from $-NH_2$) and O–H group overlap are observed in chitosan at 3283 cm^{-1} and 3198 cm^{-1}. In contrast, in COS samples, these peaks appear at 3259 cm^{-1} and 2920 cm^{-1} (Ahmad et al., 2024; Choudhary et al., 2019; Mehta et al., 2021; Ofoegbu et al., n.d.). The carbonyl (C=O) stretching vibrations from primary amides are observed at 1,651 cm^{-1} in chitosan and at 1,656 cm^{-1} in COS. The peak shifts and increased transmittance in COS samples can be attributed to their lower molecular weight and greater availability of functional groups compared to chitosan (Choudhary et al., 2019; Mehta et al., 2021; Salazar-Navarro et al., 2025).

The flexural vibrations of N-H in secondary amides are observed at 1,558 cm^{-1} and 1,537 cm^{-1} in chitosan and COS, respectively (Ahmad et al., 2024; Suryani et al., 2022). The stretching vibrations related to C-H, C-N, and C-O appear at 1,372 cm-1, 1,149 cm-1, and 955 cm-1 in chitosan samples, while in COS, they are recorded at 1,375 cm-1, 1,151 cm-1, and 1,057 cm-1 (Ahmad et al., 2024; Choudhary et al., 2019; Nguyen et al., 2023; Ofoegbu et al., n.d.). The differences between the IR spectra of chitosan and COS, along with the greater water solubility and lower molecular weight of COS, suggest a higher availability of COS to engage in chemical reactions with other molecules or materials in its surroundings, such as interacting with and modifying the SiNPs surfaces (Nguyen et al., 2023).

A schematic representation of the proposed surface structures of SiNPs, SiNPs-COS, and SiNPs-COSCu is shown in Figure 5, illustrating the progressive surface functionalization and coordination of Cu^{2+} ions with COS. This model is consistent with the FTIR data described below.

The surface modification can be verified by analyzing the IR spectra of SiNPs, SiNPs-COS, and SiNPs-COSCu, as depicted in Figure 4. SiNPs and SiNPs-COS exhibit stretching vibrations of O-H bonds, which can be attributed to the hydroxyl-rich surfaces of SiNPs in the form of silanol groups (Si-OH), along with an increase in hydroxyl groups in COS due to hydrolysis and residual water content (Hassan et al., 2024). This behavior is reflected at 3,237 cm^{-1} and 3,224 cm^{-1} in SiNPs and SiNPs-COS, respectively. Additionally, the C–H symmetric and asymmetric stretching bands observed at 2877 cm^{-1} in both SiNPs and SiNPs-COS can be attributed to residual organic chains from Silwet L-77, a siloxane-based surfactant used during the nanoparticle synthesis. Silwet L-77 contains siloxane-modified polyethylene glycol chains, which include methylene ($–CH_2–$) and methyl ($–CH_3$) groups, known to exhibit FTIR bands in the 2850–2950 cm^{-1} range (Filipkowska and Jóźwiak, 2024; Sert Çok et al., 2023). In SiNPs-COSCu, the aliphatic stretching C-H signals are intensified, exhibiting narrow peaks at wavenumbers 2,970 cm^{-1} and 2,883 cm^{-1}.

In SiNPs spectra, the non-symmetrical peaks at 1,574 cm^{-1} and 1,425 cm^{-1} can be related to bending siloxane bonds (Si-O-Si) vibrations (Hassan et al., 2024). Meanwhile, in SiNPs-COS, the signals shift to a pair of uniform peaks at the same wavelengths, which can be attributed to the N-H and C-H flexion and stretching vibrations of secondary amides due to COS deposition, as well as the maintenance of siloxane bonds in the SiNPs structure. (Ahmad et al., 2024; Hassan et al., 2024; Suryani et al., 2022). In SiNP-COSCu, the peaks associated with amide II and C-H aliphatic vibrations shift to 1,455 cm^{-1} and 1,381 cm^{-1}, exhibiting lower intensity signals. This may be more closely related to changes in the SiNPs-COSCu surfaces resulting from changes in COS organization due to Cu^{2+} ions coordination (Kumari et al., 2017; Lustriane et al., 2018; Mehta et al., 2021; Mekahlia and Bouzid, 2009).

The observed peaks at 1,039 cm^{-1}, 1,043 cm^{-1}, and 1,092 cm^{-1} in SiNPs, SiNPs-COS, and SiNPs-COS-Cu correspond to the characteristic intense signal related to the asymmetric stretching of Si-O-Si bonds (Ahmad et al., 2024; Hassan et al., 2024; Suryani et al., 2022). The observed displacement of the signal can be correlated to a progressive surface modification from hydroxyl-rich SiNPs to COS-coated SiNPs and, for instance, to Cu^{2+} ions chelated at the COS structure on the SiNPs surface.

The Si-O-Si symmetric vibrations can also be observed at 793 cm^{-1} and 788 cm^{-1} in SiNPs and SiNPs-COS, respectively (Beisl et al., 2022). The new peaks showed in SiNPs-COSCu at 877 cm^{-1} and 795 cm^{-1} could be related to the complexed formed by Cu^{2+} ions chelation by COS at SiNPs-COSCu (Choudhary et al., 2019; Fatima et al., 2024; Kumari et al., 2019; Muthukrishnan, 2015).

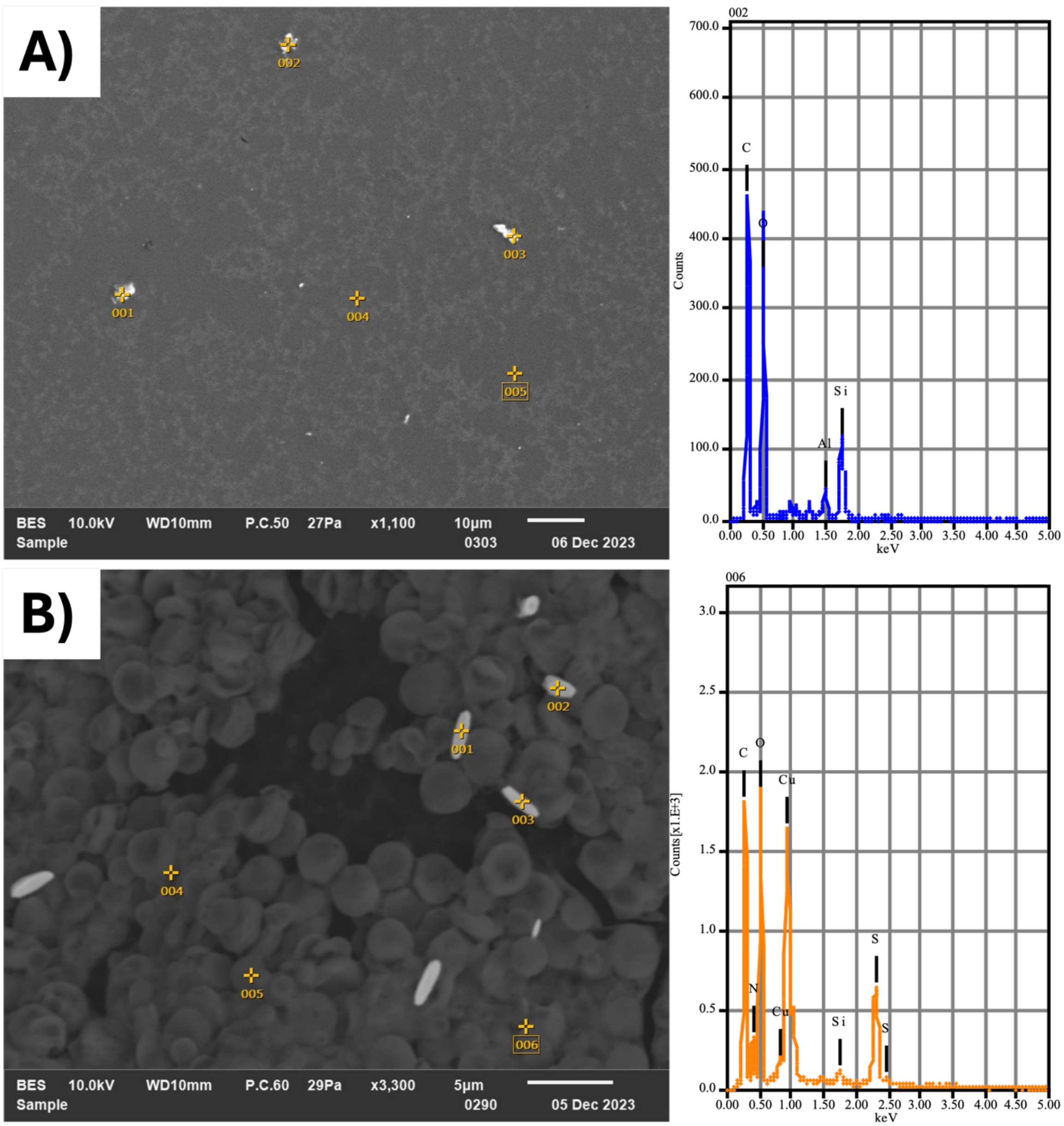


Figure 1. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis of SiNPs and SiNPs-COSCu. (A) SEM micrograph and corresponding EDS spectrum of silica nanoparticles (SiNPs) showing a homogeneous surface morphology and elemental composition mainly composed of silicon (Si), oxygen (O), and traces of carbon (C) and aluminum (Al). (B) SEM image and EDS spectrum of silica nanoparticles functionalized with chitosan oligosaccharides and copper ions (SiNPs-COSCu), revealing a rougher surface texture and aggregated globulated morphology. The EDS spectrum confirms the

presence of C, O, Si, Cu, and trace elements (N, Cl, S), associated with the chitosan and copper sulfate components.

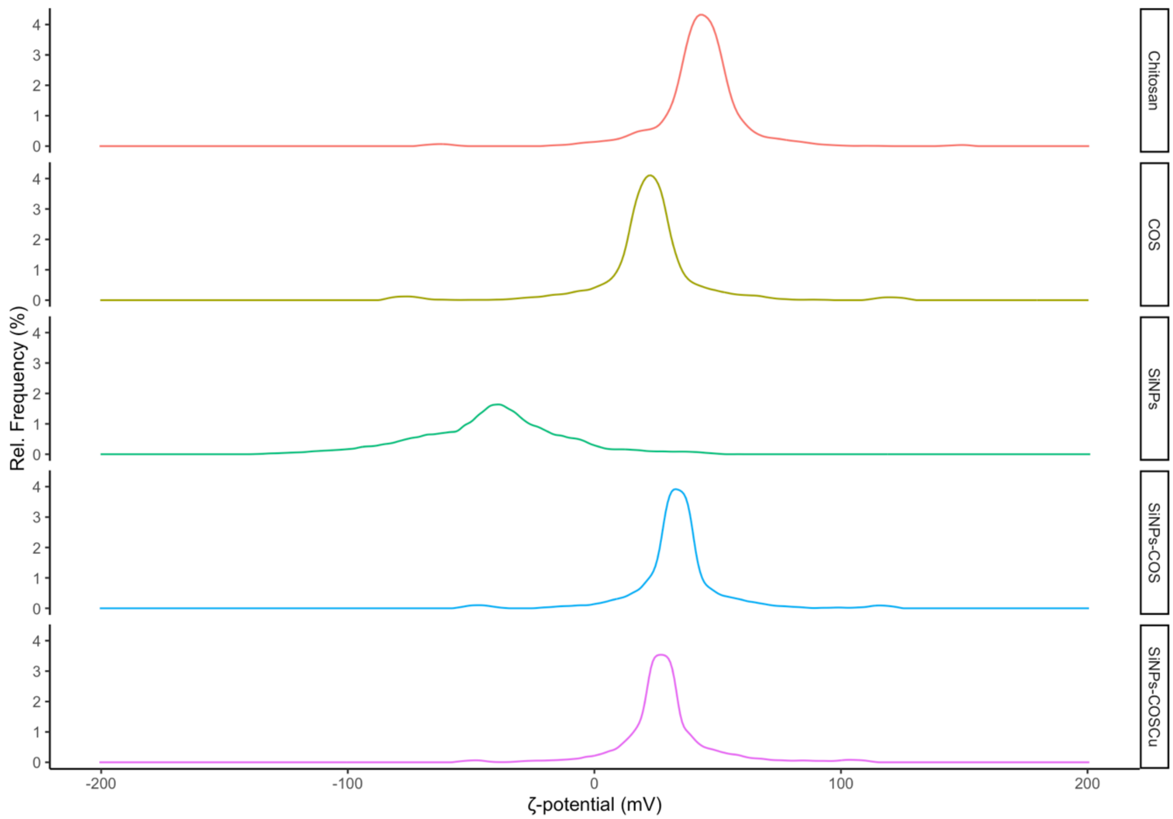


Figure 2. ζ-potential distribution profiles of chitosan, chitosan oligosaccharides (COS), silica nanoparticles (SiNPs), and functionalized nanocomposites SiNPs-COS and SiNPs-COSCu. ζ-potential was measured for each material to evaluate surface charge and colloidal stability. Chitosan and COS exhibited high positive ζ-potential values, indicative of their cationic nature. SiNPs showed a negative ζ-potential, consistent with deprotonated silanol groups on their surface. Upon functionalization with COS and COSCu, the ζ-potential of SiNPs shifted toward positive values, reflecting successful coating with cationic biopolymers and Cu^{2+} complexation. The ζ-potential trend supports the successful surface modification and potential stability of nanocomposites in aqueous suspensions.

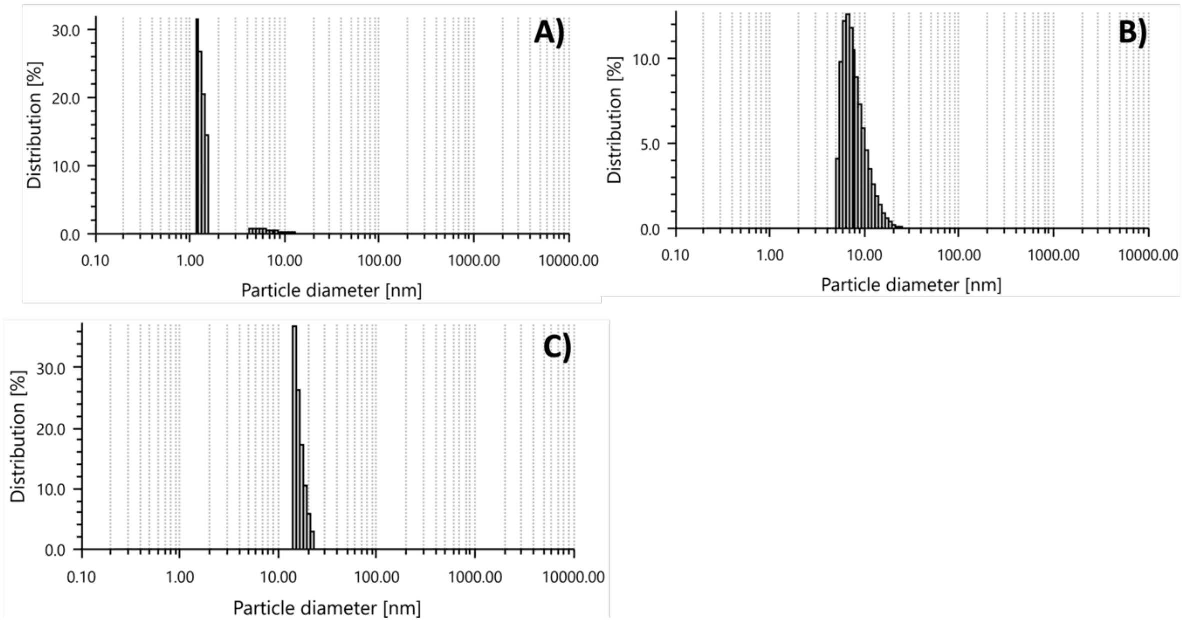


Figure 3. Particle size distribution of synthesized nanomaterials measured by dynamic light scattering (DLS) for A) Silica nanoparticles (SiNPs), B) SiNPs coated with chitosan oligosaccharides (SiNPs-COS), and SiNPs-COS doped with Cu^{2+} ions, C) SiNPs-COSCu.

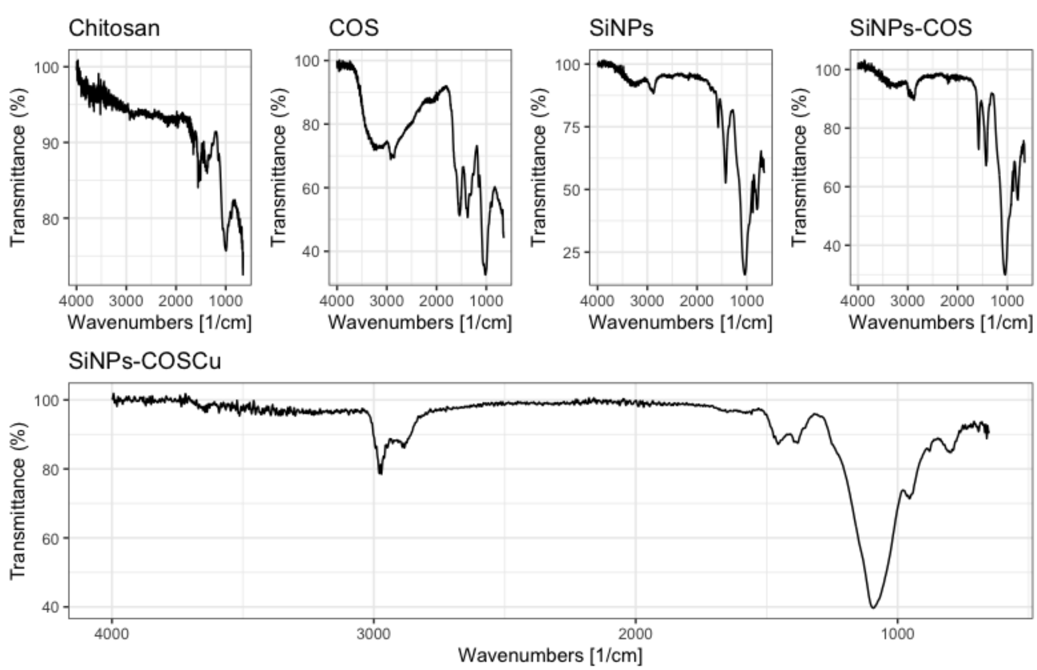


Figure 4. FTIR spectra of chitosan, chitosan oligosaccharides (COS), silica nanoparticles (SiNPs), SiNPs functionalized with COS (SiNPs-COS), and with COS and Cu^{2+} ions (SiNPs-COSCu).

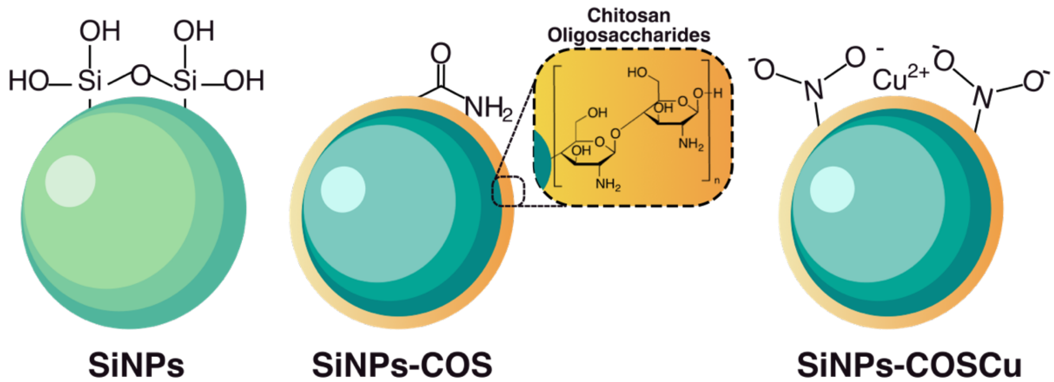


Figure 5. Schematic representation of the surface functionalization of silica nanoparticles (SiNPs), illustrating the progressive modification from hydroxylated SiNPs to chitosan oligosaccharide (COS)-coated SiNPs (SiNPs-COS) and copper-coordinated COS–SiNP hybrids (SiNPs-COSCu). The presence of Si–OH groups, COS amide functionalities, and Cu^{2+} coordination sites corresponds to the FTIR vibrational changes observed in the spectra.

3.2. Molecular response of *C. arabica* var. *Bourbon* plants

Plant-pathogen interactions are defined by the susceptibility, resistance, or tolerance of the host plant to the invading pathogen. The genetic compatibility between host genotypes and pathogen races largely determines these responses. A successful defense depends on the plant's ability to recognize the pathogen and activate defense mechanisms more rapidly than the pathogen can colonize and reproduce within the host tissues (Salazar-Navarro et al., 2024; Silva et al., 2022).

Such plant immune responses are mediated by a complex signaling network that involves perceiving external stimuli and activating pathogenesis-related (PR) genes, receptor-like proteins, and enzymes related to defense metabolism (Couttolenc-Brenis et al., 2021; Silva et al., 2022). Defense responses can be modulated and activated by several internal and external stimuli. Plants can recognize molecular patterns associated with pathogen invasion, such as pathogen-associated molecules, components of its cytoskeleton, and host-derived damage signals, including compounds found in the plant cell wall. These compounds are known as PAMPs and DAMPs, respectively (Salazar-Navarro et al., 2025; Silva et al., 2022).

PAMPs and DAMPs are recognized through transmembrane proteins (PRRs) and can activate the salicylic acid pathway and PAMPs- or DAMPs-triggered immunity (PTI). Plant defense mechanisms can be activated by exogenous molecules, which can be applied in several ways, such as foliar, radicular, and inoculation. These compounds are known as elicitors and are capable of activating defense mechanisms, inducing Systemic Acquired Resistance (SAR) (Wang et al., 2022).

Elicitors can activate and modulate plant defense responses depending on their chemical nature. Chitin derivatives, such as chitosan and chitosan oligosaccharides, are recognized by PRRs as PAMPs and can activate the responses associated with PR genes (García et al., 2021; Lidi et al., 2024). Meanwhile, Si and SiNPs can activate specific phytohormone pathways based on the source of silicon applied to the plant. Silicic acid, dissolved by SiNPs, can activate the Salicylic Acid (SA) pathway through moderate generation of ROS species, which in turn activates SA-related genes (Wang et al., 2022). Cu^{2+} ions can also activate the SA pathway by liberating ROS. However, the ROS generated in this process can be more aggressive than the ROS produced through Si-mediated photocatalytic effects with Cu^{2+} ions (Abbasirad and Ghotbi-Ravandi, 2025; Mawale et al., 2023). Excessive ROS production, particularly from high Cu^{2+} concentrations, can suppress SA-related gene expression and shift signaling toward the jasmonic acid (JA) pathway, which often acts antagonistically to SA (De et al., 2024; Haghpanah et al., 2025). Additionally, the foliar application of Cu sources is considered inefficient because Cu^{2+} ions tend to be washed away by water from the leaves (Abbasirad and Ghotbi-Ravandi, 2025).

To assess these effects in a coffee plant immune context, the expression of six defense-related genes (*PAL, PR1, PR5, β-glucanase, CaNDR1*, and *NBS-LRR*) in *Coffea arabica* was evaluated after the foliar application of SiNPs, SiNPs-COS, and SiNPs-COSCu (Figure 6). These genes are key components in various layers of the plant immune system. They are widely used as molecular markers to monitor both basal defense and systemic acquired resistance (SAR) in response to biotic stress (Couttolenc-Brenis et al., 2021; Guzzo et al., 2009; López-Velázquez et al., 2023).

This transcriptional analysis aimed to determine how silica-based nanoparticles functionalized with chitosan oligosaccharides and copper ions influence the activation of immune signaling pathways in coffee plants. The ultimate goal was to identify elicitor-capable formulations that enhance systemic defense responses and could be integrated into sustainable, preventive disease management strategies, particularly against biotrophic pathogens such as *Hemileia vastatrix*, the causal agent of coffee leaf rust.

SA synthesis and accumulation play a central role in plant responses to biotic stressors, particularly against biotrophic pathogens such as *H. vastatrix*. SA is a key signaling molecule in the activation of systemic acquired resistance (SAR) (Salazar-Navarro et al., 2024). One of the main biosynthetic routes for SA involves the phenylpropanoid pathway, where SA can be synthesized from trans-cinnamic acid via cinnamic and benzoic acid intermediates. This pathway is initiated by the expression of the *PAL* gene, which encodes phenylalanine ammonia-lyase (PAL), a key enzyme catalyzing the conversion of phenylalanine to cinnamic acid (El Houari et al., 2021; Fang et al., 2025; López-Velázquez et al., 2023).

3.2.1. PAL and NBS-LRR: Activation of SA-dependent and ETI responses

In Figure 6, RT-qPCR results show that *PAL* gene expression in SiNPs-treated plants was approximately 14.64-fold higher than in control plants, followed by SiNPs-COSCu (~4.64-fold), COS (~2.18-fold), SiNPs-COS (~1.83-fold), and the distilled water control. This pattern suggests that SiNPs treatment induced a stronger SAR response, likely through SA signaling. The *PAL* gene encodes the PAL enzyme, a key enzyme that catalyzes the initial step of the phenylpropanoid pathway, leading to the biosynthesis of SA. Additionally, PAL activity contributes to lignin production, reinforcing the plant cell wall. This structural reinforcement enhances the plant's first line of defense by strengthening mechanical barriers against pathogen invasion (El Houari et al., 2021; Fang et al., 2025; López-Velázquez et al., 2023).

The expression pattern of the *NBS-LRR* gene shown in Figure 6 mirrors that of *PAL*, with the highest expression observed in plants treated with SiNPs (~3.65-fold), followed by SiNPs-COSCu (~1.52-fold), SiNPs-COS (~1.07-fold), COS (~1-fold), and finally the distilled water control. This trend suggests an enhanced resistance response, particularly in plants treated with SiNPs and SiNPs-COSCu. The *NBS-LRR* gene, also known as a nucleotide-binding site leucine-rich repeat (NLR) gene, encodes intracellular immune receptors that are involved in effector recognition and the activation of effector-triggered immunity (ETI), which tends to result in incompatible plant-pathogen interactions that translate to resistance or tolerance to the pathogen (Angelo et al., 2023; DeYoung and Innes, 2006). Pathogen effectors are small, secreted proteins that promote infection; if not recognized promptly by host immune receptors, they enable successful colonization, leading to Effector-Triggered Susceptibility (ETS) (Salazar-Navarro et al., 2024).

These receptors coded by *NBS-LRR* are central to the gene-for-gene resistance model described by Flor, in which resistance genes (R genes) in the host detect specific pathogen effectors (Flor, 1971). In coffee, members of the LRR gene family have been associated with the S_H3 rust resistance locus. The upregulation of *NBS-LRR* in treated plants may reflect a priming effect, enhancing the plant's ability to detect and respond to *H. vastatrix* effectors more rapidly during early stages of infection (Angelo et al., 2023; Couttolenc-Brenis et al., 2021; Salazar-Navarro et al., 2024). Additionally, the activation of ETI due to the expression of NBS-LRR can induce localized resistance in the invaded cell, triggering a Hypersensitive Response (HR) characterized by localized cell death and ROS production, which effectively limits pathogen spread at the infection site. The ETI activation also induces systemic responses, including SAR activation, cell wall reinforcement, and the expression of *PR* genes (Salazar-Navarro et al., 2025, 2024).

3.2.2. *CaNDR1 and β-1,3-Glucanase: Early immune signaling and cell wall defense*

As shown in Figure 6, *CaNDR1* expression diverges from the trend observed in previous genes, with the highest expression levels detected in plants treated with SiNPs-COS (~163.01-fold), followed by SiNPs (~134.41-fold), SiNPs-COSCu (~7.05-fold), COS (~2.25-fold), and the control. *CaNDR1* belongs to the Non-Race-Specific Disease Resistance 1 (*NDR1*) gene family in *Coffea*, which plays a critical role in pattern-triggered immunity (PTI). It encodes a glycosylphosphatidylinositol (GPI)-anchored plasma membrane protein involved in amplifying defense signaling downstream of PAMP recognition. This protein is essential for a proper hypersensitive response (HR) and for establishing incompatible interactions with invading pathogens (Couttolenc-Brenis et al., 2021; Silva et al., 2022). Acting as a signaling bridge between pattern recognition receptors (PRRs) and downstream immune pathways, *CaNDR1* contributes to non-race-specific resistance mechanisms. In *Coffea arabica*, its expression has been associated with early immune responses to *H. vastatrix* in both compatible and incompatible interactions. However, NDR1-mediated signaling often depends on the presence of additional R proteins to achieve full resistance (Cacas et al., 2011; Couttolenc-Brenis et al., 2021). The elevated expression of *CaNDR1* in SiNPs-COS-treated plants suggests that this formulation effectively primes early immune signaling and enhances basal defense responses.

The expression pattern of the β-1,3-glucanase (*GLU*) gene, shown in Figure 6, follows a trend similar to *CaNDR1*, with the highest expression observed in plants treated with SiNPs (~314.45-fold), followed by SiNPs-COS (~125.03-fold), SiNPsCOSCu (~18.47-fold), COS (~5.27-fold), and the distilled water control. *GLU* encodes a hydrolytic enzyme that catalyzes the breakdown of β-1,3-glucans, which are major structural components of fungal cell walls (López-Velázquez et al., 2023). It is classified as a pathogenesis-related protein (PR-2) and plays a pivotal role in degrading invading fungal structures, facilitating the release of damage-associated molecular patterns (DAMPs) that further amplify immune signaling

(Mohammadizadeh-Heydari et al., 2024). Its expression is commonly induced as part of PAMP-triggered immunity (PTI) and also contributes to cell wall remodeling and reinforcement during pathogen attack (Yu et al., 2022; Zhang et al., 2022). The strong upregulation of *GLU* in SiNPs-treated plants suggests that this treatment promotes enhanced local antifungal defense, likely due to the synergy between silicon and chitosan oligosaccharides in activating hydrolytic enzymes. In contrast, the lower expression in SiNPs-COSCu may be related to oxidative or hormonal interferences discussed below.

Although the SiNPs-COSCu treatment resulted in strong upregulation of *PAL*, *PR1*, and *PR5*, a notable reduction in *CaNDR1* and β-1,3-glucanase (*GLU*) expression was observed compared to other treatments (Figure 6). This pattern suggests a shift in the defense strategy activated by this treatment, rather than a global suppression of the immune system.

Both *CaNDR1* and *GLU* are closely associated with early, local defense responses. *CaNDR1* functions in PAMP-triggered immunity (PTI) by amplifying defense signaling downstream of pattern recognition receptors (PRRs), while *GLU* is a hydrolytic enzyme that breaks down fungal cell walls and contributes to the release of DAMPs and strengthening of plant cell walls (Vinod W. Patil and Nilkanth S. Suryawanshi, 2025; Xu et al., 2024). Their reduced expression may be linked to the presence of Cu^{2+} ions in the SiNPs-COSCu formulation, which can generate elevated levels of reactive oxygen species (ROS).

While moderate ROS production is essential for activating PTI, excessive ROS can disrupt membrane integrity, inhibit transcription factors, and trigger a shift toward jasmonic acid (JA)-mediated signaling at the expense of salicylic acid (SA)-dependent defenses (Sahu et al., 2022; Stasińska-Jakubas and Hawrylak-Nowak, 2022). This hormonal crosstalk can result in selective suppression of defense genes related to cell wall-associated responses and immune amplification (Pieterse et al., 2012; Wang et al., 2022). Furthermore, Cu^{2+} can interfere with elicitor perception by altering chitosan conformation or affecting the surface charge of nanoparticles, reducing their capacity to trigger PAMP detection.

Therefore, the reduced expression of *CaNDR1* and *GLU* may reflect an antagonistic regulatory effect introduced by copper ions, shifting the defense response toward SAR-associated genes while dampening components of early basal immunity.

3.2.3. PR1 and PR5: SAR markers under nanoparticle stimulation

As shown in Figure 6, the expression of *PR1* was highest in plants treated with SiNPs (~34.36-fold), followed by similar expression levels in SiNPs-COS (~12.21-fold) and SiNPs-COSCu (~9.48-fold), and much lower levels in COS (~0.16-fold), with the control set as baseline. In contrast, *PR5* expression was strongest in SiNPs-COS (~33.33-fold) and SiNPs-COSCu (~30.94-fold) treatments (with similar intensity), followed by SiNPs (~25.78-fold), COS (~0.97-fold), and the control. Both *PR1* and *PR5* are hallmark genes of systemic acquired resistance (SAR) and are commonly associated with salicylic acid (SA)-mediated signaling. *PR1* is widely used as a molecular marker of SAR activation, although its exact biological function remains partially unresolved (Hönig et al., 2023; Pieterse et al., 2012). Meanwhile, *PR5*, a thaumatin-like protein (TLP), exhibits antifungal properties through membrane permeabilization and plays roles in both biotic and abiotic stress responses (Sharma et al., 2022).

The strong induction of *PR1* in SiNP-treated plants suggests that silicon alone is sufficient to trigger SA-related defense pathways, potentially through ROS-mediated priming (Acevedo et al., 2021; Greco et al., 2012). The elevated *PR5* expression in SiNPs-COS and SiNPs-COSCu may be explained by the synergistic effect of COS and copper in stimulating osmotic and oxidative stress responses, which are known to activate thaumatin-like proteins (Beatrice et al., 2017; Jia et al., 2018). Together, these results confirm that all nanocomposite treatments are capable of activating SAR-related defenses, but through distinct regulatory nodes and signaling intensities (Anisimova et al., 2021).

These results demonstrate that the foliar application of SiNPs and their functionalized variants (SiNPs-COS and SiNPs-COSCu) modulate the expression of key defense-related genes in *Coffea arabica*, affecting various layers of the plant immune system. Each nanomaterial treatment elicited distinct gene activation profiles, indicating that their chemical composition plays a central role in determining the plant's response.

SiNPs alone were particularly effective in upregulating *PAL*, *PR1*, and *NBS-LRR*, highlighting their ability to activate the SA pathway and enhance both basal and effector-triggered immunity. SiNPs-COS showed superior activation of *CaNDR1* and *PR5*, suggesting a stronger influence on early immune signaling and osmotic stress-related defenses. Interestingly, β-1,3-glucanase and *CaNDR1* were less expressed in SiNPs-COSCu-treated plants, possibly due to ROS overaccumulation caused by copper, which could inhibit components of the SA signaling pathway.

The distinct expression profiles observed suggest that SiNPs and their biopolymer or copper-functionalized forms can act as tailored elicitors, selectively enhancing different facets of the immune response. This provides valuable insights into the design of next-generation nanoparticle-based treatments for managing *Hemileia vastatrix* in coffee crops. By strategically selecting the nanoparticle composition, it may be possible to fine-tune the immune response and maximize disease resistance while minimizing phytotoxic effects.

Ultimately, these findings support the hypothesis that engineered Si-based nanocomposites function as effective modulators of plant immunity, offering a promising platform for enhancing disease resistance in perennial crops such as coffee. Further research on timing, dosage, and long-term effects will be essential to optimize their field application and efficacy under natural infection conditions.

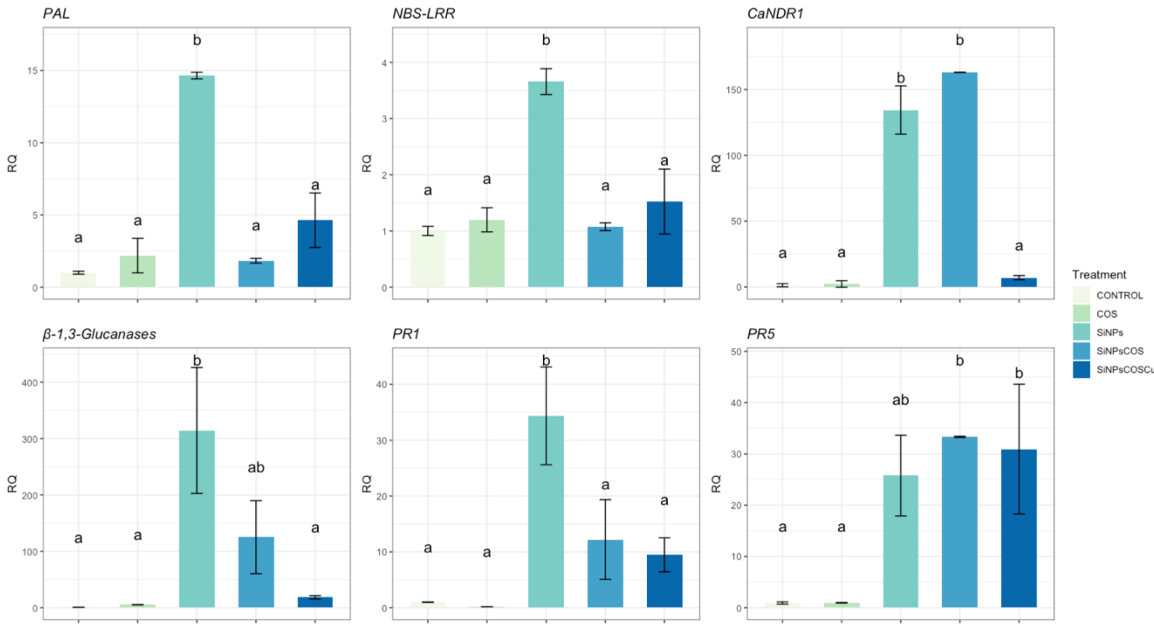


Figure 6. Relative expression (RQ) of six defense-related genes in *Coffea arabica* leaves after foliar application of nanocomposite treatments. Gene expression was analyzed by RT-qPCR three days after foliar application of the following treatments: Control (distilled water), COS (chitosan oligosaccharides), SiNPs (silica nanoparticles), SiNPs-COS (SiNPs functionalized with COS), and SiNPs-COSCu (SiNPs functionalized with COS and Cu^{2+}). Bars represent mean RQ values ± standard error (n = 3). Different letters indicate statistically significant differences among treatments (Tukey's HSD, $p < 0.05$). Upregulation of *PAL*, *NBS-LRR*, *CaNDR1*, *β-1,3-glucanases*, *PR1*, and *PR5* was associated with enhanced activation of defense pathways, particularly under SiNPs and SiNPs-COS treatments.

3.2.4. Heatmap-based clustering of immune gene expression

To better visualize the expression patterns and explore potential regulatory interactions among the studied defense-related genes, a heatmap was generated using log-transformed RT-qPCR data from all treatments (Figure 7). This clustering analysis revealed distinct gene expression signatures associated with the chemical composition of each nanomaterial.

The heatmap shows a close cluster between SiNPs and SiNPs-COS treatments, suggesting a strong similarity in the expression profiles of *PR1*, *PR5*, *GLU*, and *CaNDR1*. This pattern reflects a robust activation of SA-dependent signaling and basal defense mechanisms in response to both formulations. However, subtle differences in the expression of genes such as *PAL* and *NBS-LRR* may be attributed to the COS coating, which could delay the degradation of SiNPs into silicic acid. This may result in a more sustained immune activation in plants treated with COS-functionalized SiNPs due to the gradual release of the active elicitor.

By contrast, SiNPs-COSCu clustered apart, showing a differential expression profile. While *PR1* and *PR5* remained strongly expressed, the marked reduction in *GLU* and *CaNDR1* expression suggests a shift from local basal defenses toward systemic responses, possibly due to oxidative stress induced by Cu^{2+} ions. COS alone, along with the control, formed the lowest-expression cluster, indicating minimal immune activation.

These clustering patterns support the individual gene expression results and highlight the coordinated activation or suppression of specific immune pathways depending on the chemical nature of each nanocomposite.

Interestingly, COS-treated plants showed an expression pattern that closely resembled the control, which contrasts with the expected immune activation typically associated with chitin derivatives. Given that chitosan oligosaccharides are recognized as PAMPs, this may reflect a dose-dependent or structural limitation in elicitor recognition. In contrast, engineered Si-based nanoparticles functionalized with COS and/or Cu^{2+} appear capable of inducing sustained and selective immune responses, likely due to the combined and gradual release of elicitor-active molecules such as silicic acid, COS, and copper ions.

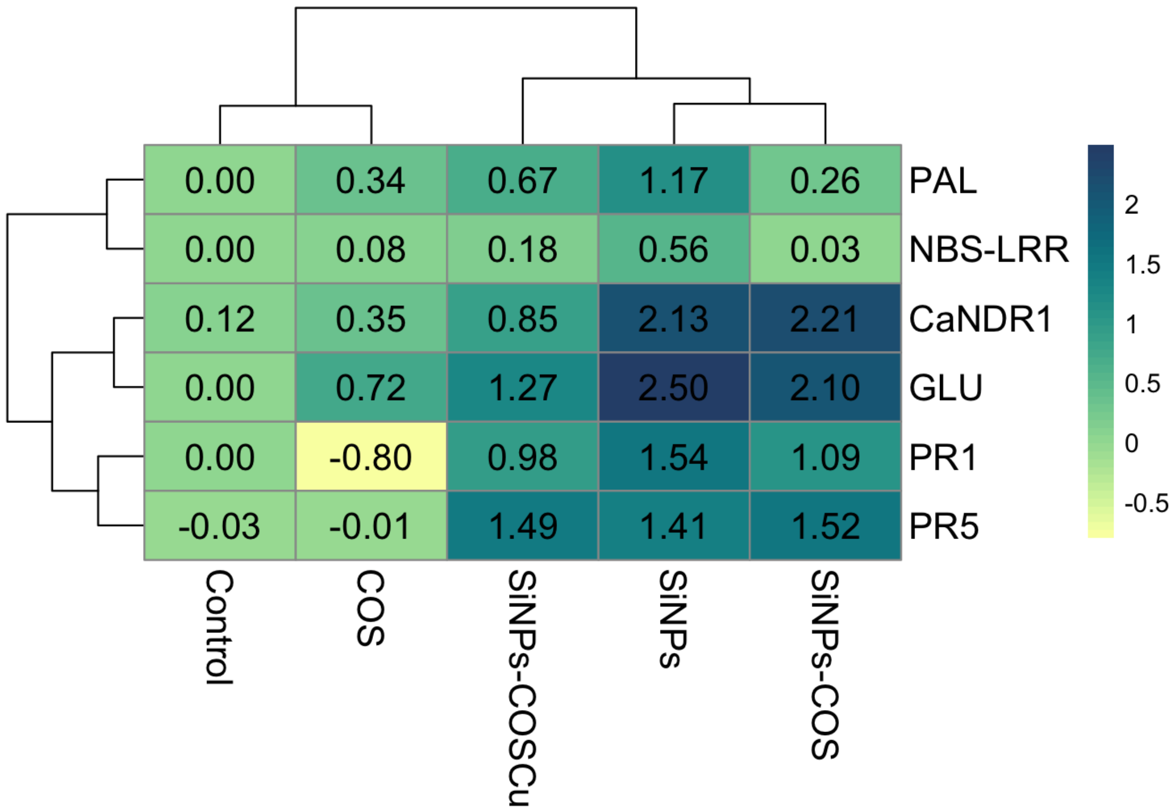


Figure 7. Heatmap and hierarchical clustering of relative gene expression in *Coffea arabica* leaves under different nanoparticle treatments. The heatmap shows the normalized relative expression values (log_2-transformed and z-score standardized) of six defense-related genes (*PAL*, *NBS-LRR*, *CaNDR*1, *GLU [β-1,3-glucanases]*, *PR1*, *PR5*) in response to five foliar treatments: Control, COS, SiNPs-COSCu, SiNPs, and SiNPs-COS. Both genes and treatments were clustered using Euclidean distance and complete linkage. High expression levels are represented in blue, and low expression levels in yellow. Clustering reveals two main treatment groups: one comprising nanoparticle-based treatments (SiNPs, SiNPs-COS, SiNPs-COSCu) and another with Control and COS, suggesting that nanocomposites significantly modify transcriptional defense profiles in coffee leaves.

3.3. Specific Enzymatic Activity response of *C. arabica* var. *Bourbon* plants

In Figure 8, the specific enzymatic activity—calculated relative to total soluble protein content (SPC)—is presented for enzymes associated with salicylic acid (SA)-mediated defense and oxidative stress regulation. These include PAL, β-1,3-glucanase, and chitinase, as well as the antioxidant enzymes peroxidase (POD) and catalase (CAT).

Interestingly, in contrast to the gene expression results, COS-treated plants exhibited higher enzymatic activity of PR2- and PR3-related proteins, such as β-1,3-glucanase and chitinase with values of 137.94 ± 13.36 and 19.90 ± 2.17 μmol min^{-1} μg^{-1} protein, respectively (Salazar-Navarro et al., 2025, 2024). This suggests that COS may promote the activation or stabilization of pre-existing defense proteins, possibly due to its high biocompatibility or elicitor potential at the post-transcriptional level (dos Santos and Franco, 2023). This discrepancy between transcriptional and enzymatic responses may reflect differences in recognition kinetics, mRNA-protein turnover, or elicitor-triggered signaling cascades.

Moreover, this enhanced enzymatic activity may be linked to reactive oxygen species (ROS) generation triggered by pattern-triggered immunity (PTI). COS, as a chitin-derived molecule, is recognized by pattern recognition receptors (PRRs), leading to oxidative burst and the subsequent activation of antioxidant defenses, including POD and CAT (dos Santos and Franco, 2023). The upregulation of these enzymes in COS-treated plants supports this interpretation and highlights its role as a priming agent capable of activating multiple layers of immunity.

In contrast, PAL activity aligned more closely with its gene expression profile, showing elevated levels in SiNPs- and SiNPs-COS-treated plants (4.80 ± 0.23 and 5.10 ± 0.08 μmol min^{-1} μg^{-1} protein, respectively), with a slight reduction in the SiNPs-COSCu group (3.65 ± 0.13 μmol min^{-1} μg^{-1} protein). This suggests that Si-based nanomaterials effectively stimulate SA biosynthesis through both de novo PAL gene expression and post-translational activation, potentially leading to a rapid and sustained activation of systemic acquired resistance (Du et al., 2022).

In summary, the enzymatic activity profiles observed in treated *Coffea arabica* plants support the notion that nanocomposites can modulate both transcriptional and post-transcriptional defense responses. While Si-based formulations, particularly SiNPs and SiNPs-COS, were effective in promoting PAL-associated SA biosynthesis, COS alone appeared to enhance the accumulation or activation of defense enzymes such as β-1,3-glucanase and chitinase, likely through PTI-related mechanisms (Fauteux et al., 2005; Jia et al., 2018). The increased activities of POD and CAT in COS and SiNPs-COSCu treatments further suggest the occurrence of oxidative stress and highlight the plant's effort to mitigate ROS accumulation (Song et al., 2023). These findings emphasize that each nanomaterial triggers a distinct defense strategy, with varying impacts on both enzymatic defense capacity and cellular redox homeostasis.

To further explore the physiological consequences of these treatments, we next assessed membrane integrity and oxidative damage indicators, including electrolyte leakage (EL) and membrane stability (MS), as markers of cellular stress and compatibility with nanomaterial application.

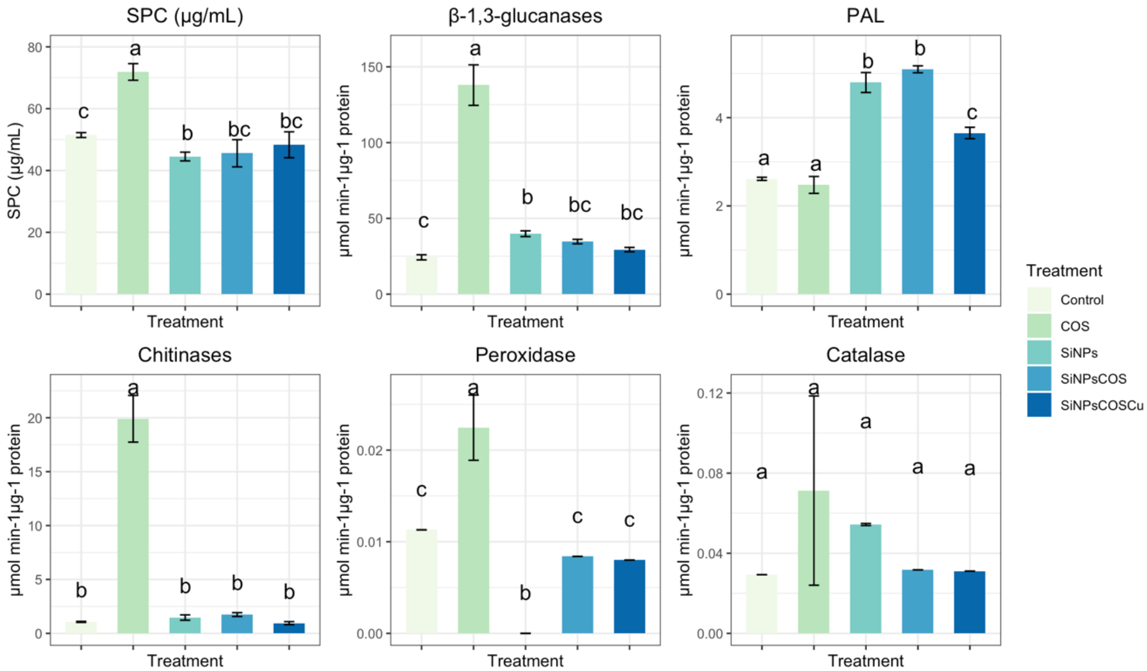


Figure 8. Specific enzymatic responses in *Coffea arabica* leaves following foliar application of nanocomposites. Bar plots show the effect of five treatments (Control, COS, SiNPs, SiNPs-COS, and SiNPs-COSCu) on six biochemical parameters: soluble phenolic compounds (SPC, µg/mL) and the activity of five enzymes—β-1,3-glucanases, phenylalanine ammonia-lyase (PAL), chitinases, peroxidase, and catalase. Enzyme activities are expressed as µmol min^{-1} μg^{-1} protein. Data are presented as mean ± SE. Different letters above bars indicate significant differences among treatments (Tukey's test, $p < 0.05$). Treatments with SiNPs and SiNPs-COS led to enhanced PAL activity, while COS alone induced the highest SPC content and enzymatic activity of chitinases and peroxidase. Nanocomposite treatments maintained moderate to high enzymatic responses without excessive oxidative stress.

3.4. Membrane integrity and oxidative stress indicators

To assess the physiological impact of nanocomposite application on *Coffea arabica* plants, membrane integrity was evaluated using electrolyte leakage (EL) and membrane stability (MS) assays (Figure 9). These parameters are widely recognized as indicators of oxidative damage and cellular compatibility with biotic or abiotic stimuli (Rasheed et al., 2022; Sieprawska et al., 2024).

SiNPs and SiNPs-COS treatments exhibited low EL values and high MS percentages, reflecting minimal membrane damage and good biocompatibility. Specifically, SiNPs-treated plants showed EL of 17.99 ± 2.39% and MS of 82.01 ± 2.39%, while SiNPs-COS-treated plants showed EL of 12.95 ± 0.06% and MS of 87.05 ± 0.06%. In contrast, SiNPs-COSCu-treated plants displayed higher EL (21.15 ± 12.96%) and lower MS (78.85 ± 12.96%), indicating a trend toward membrane destabilization and potential cytotoxic effects. These findings align with the activity of antioxidant enzymes (POD and CAT) observed in each treatment, suggesting that SiNPs and SiNPs-COS maintain a balanced ROS detoxification dynamic, while Cu^{2+} in SiNPs-COSCu may contribute to oxidative imbalance.

Interestingly, COS-treated plants maintained high MS values despite their strong enzymatic activity of glucanase, chitinase, and POD, supporting the hypothesis that COS elicits defense responses without causing substantial oxidative damage. This highlights the functional versatility of COS as an elicitor that can activate immune responses while maintaining cellular homeostasis (Elkarmout et al., 2022; Hao et al., 2023; Yang et al., 2009).

Together, these results confirm that SiNPs-based nanomaterials differ in their ability to modulate oxidative stress and membrane integrity, depending on their surface chemistry. While COS and SiNPs-COS preserve membrane function, SiNPs-COSCu may trigger an overactivation of ROS-related responses, potentially compromising early defense layers such as *GLU* expression and *CaNDR1* signaling, as observed previously.

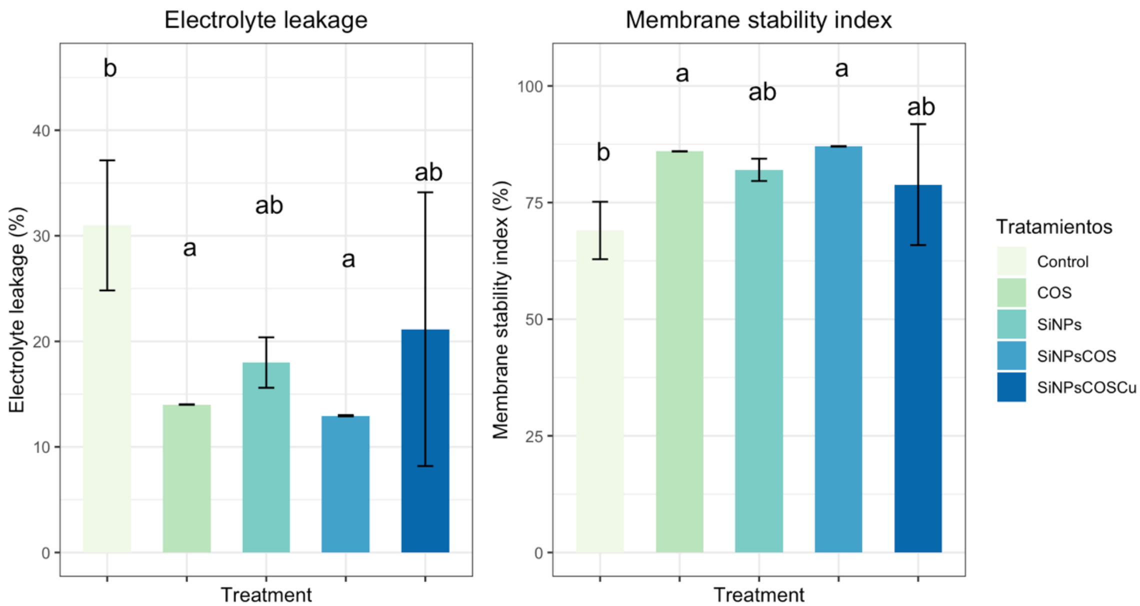


Figure 9. Electrolyte leakage and membrane stability index in *Coffea arabica* leaves under different treatments. Bar plots show the effect of foliar treatments (Control, COS, SiNPs, SiNPs-COS, and SiNPs-COSCu) on two indicators of cell membrane integrity: electrolyte leakage (%) and membrane stability index (%). Data are expressed as mean ± SE. Different letters denote statistically significant differences among treatments (Tukey's test, $p < 0.05$). Treatments with nanocomposites (especially SiNPs and SiNPs-COS) significantly reduced electrolyte leakage compared to the control, indicating enhanced membrane protection. Likewise, the membrane stability index increased in plants treated with nanoparticles, supporting the protective effect of nanocomposites on cellular structures.

4. Conclusions

The present study demonstrates that the surface functionalization of silica nanoparticles (SiNPs) with chitosan oligosaccharides (COS) and copper ions (Cu^{2+}) enhances their physicochemical properties and biological efficacy. FTIR analyses confirmed the progressive incorporation of functional groups associated with COS and Cu^{2+} chelation, while DLS and ζ-potential measurements indicated improved colloidal stability and surface charge modification in SiNPs-COS and SiNPs-COSCu formulations.

These structural modifications translated into significant biological effects, as evidenced by the differential expression of key defense-related genes in *Coffea arabica*. SiNPs-COSCu elicited the strongest induction of SAR-related markers (*PR1*, *PR5*), recognition and signaling genes (*NBS-LRR*, *CaNDR1*), and metabolic defense components (*PAL*, *β-glucanase*). This suggests that functionalized nanoparticles act as effective elicitors of the plant immune system, likely by mimicking or amplifying pathogen-associated signals through optimized nanoscale interactions.

Overall, the integration of COS and Cu into the SiNPs surface not only improves the nanoparticles' physicochemical profile but also enhances their bioactivity *in planta*. These findings support the potential application of functionalized SiNPs as sustainable tools for crop protection and immune stimulation in coffee plants, particularly as a preventive strategy to enhance early defense responses before pathogen establishment.

5. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

6. Author Contributions

SA: Writing – original draft. RV: Writing – review & editing. JJ: Writing – review & editing. VB: Writing – review & editing DD: Writing – review & editing TO: Writing – review & editing BU: Writing – review & editing. GD: Writing – review & editing.

7. Acknowledgments

The author Salazar-Navarro (CVU 968662) acknowledges the Secretaria de Ciencia, Humanidades, Tecnologías e Innovación (SECIHTI) for the support offered during his postgraduate studies in the Doctorado en Ciencias Agrícolas at the Instituto de Ciencias Agrícolas in the Universidad Autónoma de Baja California.

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CAPÍTULO 8. CONCLUSIONES Y PERSPECTIVAS FUTURAS

1. Conclusiones generales

La presente tesis permitió demostrar el potencial de los nanomateriales funcionalizados como herramientas elicitoras en el cultivo de *Coffea arabica* var. *Bourbon* frente a la amenaza de *Hemileia vastatrix*. A partir de la síntesis y caracterización de nanopartículas de sílice (SiNPs) funcionalizadas con oligómeros de quitosano (COS) y iones cúpricos (Cu^{2+}), se logró inducir de manera efectiva respuestas bioquímicas y moleculares asociadas a mecanismos de defensa en las plantas, sin evidencias de fitotoxicidad.

Las evaluaciones bioquímicas demostraron una activación significativa de enzimas clave como PAL, β-1,3-glucanasa, quitinasa, catalasa y peroxidasa, particularmente en tratamientos con SiNPs-COS y SiNPs-COSCu. A nivel molecular, se registró una inducción de genes relacionados con rutas de resistencia sistémica adquirida (SAR) y respuesta hipersensible (HR), como *PR1, PR5, CaNDR1* y *NBS-LRR*. Además, las evaluaciones con discos foliares inoculados mostraron un efecto preventivo de los tratamientos, evidenciado por una menor germinación de urediniosporas, menor daño celular y mayor integridad de membrana.

Los resultados obtenidos respaldan la hipótesis planteada y confirman que los nanocompuestos desarrollados pueden actuar como inductores tempranos de defensa en café. Estos hallazgos posicionan a los nanoelicitores evaluados como una estrategia biotecnológica prometedora para el manejo sostenible de la roya del café.

2. PERSPECTIVAS

- **a.** Se recomienda la validación de los nanocompuestos funcionalizados en ensayos a campo abierto y en condiciones productivas reales, bajo diferentes contextos agroclimáticos.
- **b.** Actualmente se encuentran en desarrollo ensayos de validación funcional en colaboración con el Instituto Hondureño del Café

(IHCAFÉ), los cuales permitirán evaluar la eficacia biológica de los tratamientos bajo condiciones de infección controlada con *H. vastatrix*.

c. Futuras investigaciones podrían explorar la sinergia entre estos nanocompuestos y microorganismos benéficos, así como su impacto en el microbioma foliar y rizosférico del cafeto.

d. Se sugiere el diseño de formulaciones comerciales que aprovechen la estabilidad coloidal y la biocompatibilidad de los nanomateriales desarrollados, con miras a su escalamiento industrial como bioinsumos agrícolas.

e. Previo a su escalamiento y aplicación agrícola, se sugiere la realización de estudios relacionados con posibles efectos ecotóxicos de los nanotratamientos, por ejemplo, su impacto en el microbioma y posible bioacumulación.

f. Finalmente, este enfoque puede ser transferible a otros cultivos de importancia económica afectados por enfermedades fúngicas, ampliando su aplicabilidad dentro de la nanotecnología agrícola.

ANEXOS

1. PONENCIAS EN CONGRESOS NACIONALES E INTERNACIONALES


Certificate of Appreciation

Awarded to

Prof./Dr./Mr. Alexis Alejandro Salazar Navarro, Autonomous University of Baja California, Mexico

for participating and presenting on Biochemical Responses of Coffea Arabica Var. Borbon to Foliar Exposure of Copper Doped Silica Chitosan Oligosaccharides Nanoparticles

at

10th Nanotech & Nanomaterials Research Conference

held on November 06-08, 2024 | London, UK

Nano London-2024
November 06-08, 2024 | London, UK

Kenneth Blum, PhD, DHL
President
USG-United Scientific Group
(A non-profit organization)


Certificate of Appreciation

Awarded to

Prof./Dr./Mr./Mrs./Ms. Alexis Alejandro Salazar Navarro, Autonomous University of Baja California, Mexico

for participating and presenting on Synthesis and Functionalization of Silica Nanoparticles with Silver Ions as a Hydrophobic and Antibacterial Fabric Coating

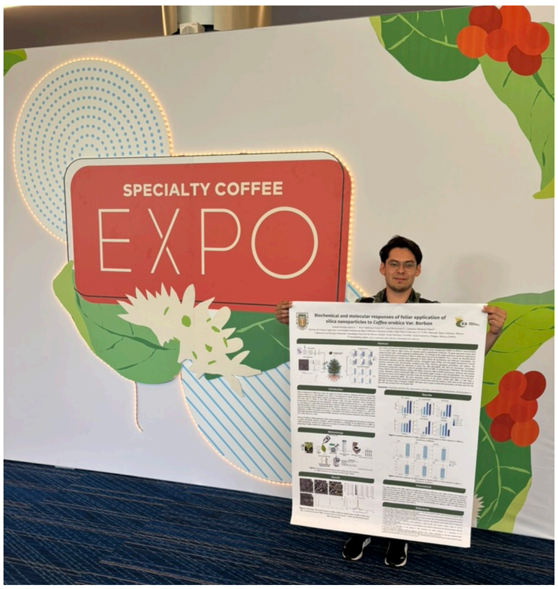
at

10th Nanotech & Nanomaterials Research Conference

held on November 06-08, 2024 | London, UK

Nano London-2024
November 06-08, 2024 | London, UK

Kenneth Blum, PhD, DHL
President
USG-United Scientific Group
(A non-profit organization)




III SIMPOSIO INTERNACIONAL DE PARASITOLOGÍA AGRÍCOLA

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE CIENCIAS AGRÍCOLAS

Otorgan la presente

CONSTANCIA

A

MC. Alexis A. Salazar Navarro

Por su participación como PONENTE con el trabajo titulado:
Evaluación de nanopartículas de cobre y sílice para el control de *Phenacoccus solenopsis* (piojo harinoso)
En modalidad de presentación oral

Dr. Daniel González Mendoza
Director
Instituto de Ciencias Agrícolas

Mexicali, Baja California, 26 abril de 2023
"Por la realización plena del ser"


UNIVERSIDAD AUTÓNOMA DE CHIAPAS
FACULTAD DE CIENCIAS AGRONÓMICAS
CAMPUS V

OTORGA EL PRESENTE
RECONOCIMIENTO AL C:

M.I. ALEXIS ALEJANDRO SALAZAR NAVARRO

Por la impartición de la conferencia: "NANOPARTÍCULAS Y SU IMPACTO EN CULTÍVOS AGRÍCOLAS", realizado el 22 de enero de 2025, en el marco del ciclo de conferencias: Estrategias Integradas al Desarrollo Agroambiental, en colaboración con el Programa Académico de Ingeniero en Desarrollo Agroambiental.

Villaflores, Chiapas; 22 de enero de 2025.

"POR LA CONCIENCIA DE LA NECESIDAD DE SERVIR"

M.C. CARLOS ALBERTO VELÁZQUEZ SANABRIA
DIRECTOR

M.C. FREDY DELGADO RUIZ
SECRETARIO ACADÉMICO

CAMPUS V


La organización del

XXVI SIMPOSIO LATINOAMERICANO DE CAFICULTURA

Otorga el presente diploma a:

Alexis Alejandro Salazar Navarro

Por su destacada participación en las actividades académicas, técnicas y de intercambio de conocimientos llevadas a cabo durante el XXVI Simposio Latinoamericano de Caficultura, realizado del 15 al 18 de julio del 2025.

Su compromiso y presencia contribuyeron al fortalecimiento del sector cafetalero y al desarrollo sostenible de la caficultura.

Daniel Dubón
Secretario Ejecutivo
PROMECAFE

Mauricio Sansivirini
Presidente
Instituto Salvadoreño del Café (ISC)

San Salvador, El Salvador 2025.

2. ACTIVIDADES DE DIVULGACIÓN DE LA CIENCIA

Universidad Autónoma de Baja California

UABC Radio

INSTITUTO DE CIENCIAS AGRÍCOLAS

"2025, año del Turismo Sostenible como impulsor del Bienestar Social y Progreso"

Mexicali, Baja California, a 11 de agosto del 2025

Asunto: Constancia de participación en

programa radiofónico "Agricultura y Ciencia"

MC. Alexis Alejandro Salazar Navarro

P R E S E N T E.-

La Universidad Autónoma de Baja California, a través del Instituto de Ciencias Agrícolas, reconoce y agradece la valiosa participación del MC. Alexis Salazar Navarro en el **programa radiofónico "Agricultura y Ciencia"**, donde se abordó el tema **"La aplicación de nanopartículas en agronomía",** transmitido desde las instalaciones de **UABC Radio y Televisión Digital** y a través de internet por radio.uabc.edu.mx, el día **28 de agosto del 2025**.

Su contribución ha sido fundamental para la **divulgación del conocimiento científico y tecnológico relacionado con el sector agropecuario**, en beneficio de la comunidad agrícola y el público en general.

Aprovechamos la ocasión para enviarle un cordial saludo y reiterar nuestro reconocimiento por su valioso aporte.

ATENTAMENTE
"POR LA REALIZACIÓN PLENA DEL SER"

DR. DANIEL GONZÁLEZ MENDOZA
Director del Instituto de Ciencias Agrícolas

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE CIENCIAS AGRÍCOLAS

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE CIENCIAS AGRÍCOLAS

Otorga la presente

CONSTANCIA

a

M.C. Alexis Salazar Navarro

Por su participación como **organizador** del evento *"Recorrido de Campo sobre Cultivos Agrícolas Productores de Antioxidantes y su Uso Biotecnológico"* dirigido a estudiantes del Valle de Mexicali, llevado a cabo el día 12 de junio de 2025 .

"POR LA REALIZACIÓN PLENA DEL SER"

Dr. Daniel González Mendoza
Director

Mexicali; Baja California, 12 de junio de 2025.

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE CIENCIAS AGRÍCOLAS

Otorga la presente

CONSTANCIA

a

M.C. Alexis Salazar Navarro

Por su participación como **Apoyo técnico** del evento *"Recorrido de Campo en Parcela Demostrativa de Cebada Maltera"* dirigido a productores del Valle de Mexicali y comunidad estudiantil, llevado a cabo el día 2 de mayo de 2025 .

"POR LA REALIZACIÓN PLENA DEL SER"

UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
INSTITUTO DE CIENCIAS AGRÍCOLAS DIRECCIÓN

Dr. Daniel González Mendoza
Director

Mexicali; Baja California, 2 de mayo de 2025.

3. PARTICIPACIÓN EN CONFERENCIAS Y PONENCIAS MAGISTRALES

UNIVERSIDAD AUTÓNOMA DE CHIAPAS
FACULTAD DE CIENCIAS AGRONÓMICAS *CAMPUS* V
DIRECCIÓN

Villaflores, Chiapas
20 de enero de 2025
FCACV/D/021/2025

M.I. Alexis Alejandro Salazar Navarro
Universidad Autónoma de Baja California
P r e s en t e

Estimado MI. Salazar:

Por este medio me permito saludarlo deseándole éxito en sus actividades personales y laborales; así mismo hago llegar a usted la presente invitación para que nos acompañe al ciclo de conferencias: Estrategias Integradas al Desarrollo Agroambiental; así también solicitamos su valiosa colaboración como ponente en la conferencia: **NANOPARTÍCULAS Y SU IMPACTO EN CULTÍVOS AGRÍCOLAS**, a realizarse en la sala Ing. Héctor Saucedo Martínez de esta Facultad, el día miércoles 22 de enero a las 10:00 horas.

Sin más por el momento, le agradezco su valiosa colaboración.

FACULTAD DE CIENCIAS AGRONOMICAS
UNIVERSIDAD AUTONOMA DE CHIAPAS
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A t e n t a m e n t e
"Por la Conciencia de la Necesidad de Servir"

M. C. Carlos Alberto Velázquez Sanabria
D i r e c t o r

C. c. p. Archivo

CAVS*marh

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Tel/Fax 01 965 65 2 14 77 y 5 32 72 www.unach.mx, fac.agronomicas@unach.mx

UNIVERSIDAD AUTÓNOMA DE CHIAPAS
ESCUELA DE SISTEMAS ALIMENTARIOS
SIMPOSIO INTERNACIONAL ¨SISTEMAS ALIMENTARIOS MESOAMERICANOS¨

SIAL ESCUELA DE SISTEMAS ALIMENTARIOS UNACH

Tapachula, Chiapas a 19 de febrero de 2025
Oficio No. D-SiAL/SISAS/027/2025

Mtro. Alexis Salazar Navarro
Profesor Investigador
Universidad Autónoma de Baja California
P r e s e n t e .-

Reconocido Mtro. Alexis Salazar

Como parte de las dinámicas académicas, de internacionalización y de socialización del conocimiento de la Escuela de Sistemas Alimentarios de la Universidad Autónoma de Chiapas (SIAL-UNACH) y del desarrollo de las líneas de investigación del Cuerpo Académico ¨Sistemas Alimentarios¨, tenemos el honor de invitarlo al PRIMER SIMPOSIO INTERNACIONAL DE SISTEMAS ALIMENTARIOS MESOAMERICANOS, ha realizarse los días 24 y 25 de abril de 2025, en la ciudad de Tapachula, Chiapas, México.

Por lo que nos complacerá tenerlo como PONENTE MAGISTRAL con el tema:

¨ Nanoparticulas de silicio y sus modificaciones como posibles biofortificantes y alternativas para el control de fitopatógenos de Coffea arabica ¨

Será un gusto poder interactuar y generar un espacio de reflexión, análisis y coordinar posibles acciones de interés común entorno a los Sistemas alimentarios.

Esperando contar con lo solicitado en pro de los logros proyectados, me remito a sus distinguidas consideraciones.

ATENTAMENTE
"POR LA CONCIENCIA DE LA NECESIDAD DE SERVIR"

ING. Y M.C. PEDRO RENÉ BODEGAS VALERA
DIRECTOR DE LA SiAL-UNACH

UNIVERSIDAD AUTÓNOMA DE CHIAPAS
SIAL. C-IV
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1ER SIMPOSIO INTERNACIONAL DE SISTEMAS ALIMENTARIOS MESOAMERICANOS

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Con copia para:
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DRA. SANDRA ISABEL RAMÍREZ GONZÁLEZ. Responsable General del Simposio Internacional en Sistemas Alimentarios Sostenibles.
Archivo/minutario.

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Oficina: (962) 62 8 44 72;(962) 62 8 44 98; Correo electrónico: direccion.sial@unach.mx, pedro.bodegas@unach.mx
unach.mx

4. FORMACIÓN DE CAPITAL HUMANO

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TECNOLÓGICO NACIONAL DE MÉXICO

Instituto Tecnológico de Tuxtla Gutiérrez

INSTITUTO TECNOLOGICO DE TUXTLA GUTIERREZ

"ELABORACION Y CARACTERIZACION DE FORMULACIONES FARMACEUTICAS ADICIONADAS CON NANOPARTÍCULAS Y EXTRACTO PETIVERIA ALLIACEA L."

COMO REQUISITO PARA OBTENER EL TITULO DE:
INGENIERÍA BIOQUÍMICA

PRESENTA:
ALEJANDRA JONAPA JUAREZ

DIRECTOR TESIS:
DRA. ROSA ISELA CRUZ RODRÍGUEZ

CO-DIRECTOR:
MTRO. ALEXIS ALEJANDRO SALAZAR NAVARRO

Tuxtla Gutiérrez, Chiapas
Julio 2024

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SECRETARÍA DE EDUCACIÓN PÚBLICA

TECNOLÓGICO NACIONAL DE MÉXICO

Instituto Tecnológico de Tuxtla Gutiérrez

INSTITUTO TECNOLÓGICO DE TUXTLA GUTIÉRREZ

TRABAJO PROFESIONAL
COMO REQUISITO PARA OBTENER EL TÍTULO DE:
INGENIERA BIOQUÍMICA

MEDIANTE:
OPCION (TESIS PROFESIONAL)

TÍTULO
"EVALUACIÓN DEL IMPACTO DE NANOPARTÍCULAS DE DIÓXIDO DE TITANIO EN EL CRECIMIENTO Y PARÁMETROS BIOQUÍMICOS DE PLÁNTULAS DE CAFÉ (*Coffea arabica* L.) EN CONDICIONES DE HIDROPONÍA"

QUE PRESENTA:
Rosa María Gómez Santiz

DIRECTOR DE TESIS:
Dr. Víctor Manuel Ruíz Valdiviezo

CODIRECTOR DE TESIS:
M.I. Alexis Alejandro Salazar Navarro

Tuxtla Gutiérrez, Chiapas

Mayo, 2025

5. PUBLICACIÓN DE CAPÍTULOS DE LIBROS

3.14 Síntesis de nanopartículas de cobre con extracto de *Aloe vera* para control de *Tribolium castaneum*

Salazar-Navarro Alexis A.[1,2], Valdez-Salas Benjamín[3], Rivera-Reyna Nallely E[2]., Ail-Catzim Carlos Enrique[1] y González-Mendoza Daniel[1]

[1]Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California (ICA–UABC). Carretera a Delta S/N C.P. 21705, Ejido Nuevo León, Baja California, México. alexis.salazar@uabc.edu.mx. [2]Industrias Bioquim, Carretera Aeropuerto S/N Km 3. 21600, Mexicali, Baja California, Mexico. [3]Instituto de Ingeniería de la Universidad Autónoma de Baja California, Calle de la Normal S/N y Boulevard Benito Juárez, 21100, Mexicali, Baja California, México.

CAPITULO

SÍNTESIS, CARACTERIZACIÓN Y APLICACIONES DE NANOPARTÍCULAS EN LA AGRICULTURA.

SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS OF NANOPARTICLES IN AGRICULTURE.

Alexis A. Salazar-Navarro[1*], José G. Joya-Dávila[1], Leslie A. Serrano-Gómez[2], Carlos Moreno-Cruz[1], Daniel González-Mendoza[1]

[1]Laboratorio de biotecnología vegetal, Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California

[2]Laboratorio 12 del polo tecnológico, Tecnológico Nacional de México Campus Tuxtla Gutiérrez

*Autor de correspondencia: alexis.salazar@uabc.edu.mx

RESUMEN

El uso excesivo de compuestos químicos en la agricultura como fertilizantes, pesticidas, promotores de crecimiento, entre otros, ha generado daños ambientales en el suelo y mantos acuíferos. Por lo tanto una alternativa viable y ecológica es el uso de nanopartículas, principalmente por el aprovechamiento de sus propiedades fisicoquímicas que le confieren mayor efectividad y eficiencia que su contra parte macroscópica. Las rutas o metodología empleadas para la síntesis de los nanomateriales dependen estrictamente del objetivo de la aplicación del tratamiento, lo que sugiere un diseño de síntesis con mayor precisión. Los nanomateriales para implementarse pueden obtenerse a partir de rutas química, físicas y biológicas, mientras que su composición puede ser de origen orgánico, inorgánico, basado en estructuras de carbono o como una combinación de los materiales precursores. Dentro de sus principales ventajas, se encuentra la posibilidad de modificar el nanomaterial para que pueda intervenir en diversas aplicaciones según sea requerido, esto a partir de encapsulados de compuestos

6. PUBLICACIÓN DE ARTÍCULOS COMO PRIMER AUTOR

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Synthesis of silica chitosan oligosaccharides nanoparticles

Salazar-Navarro, Alexis A.[1]**; Rivera-Reyna, Nallely E.**[2]**; González-Mendoza, Daniel**[1*]

[1] Universidad Autónoma de Baja California Instituto de Ciencias Agrícolas de la (ICA-UABC). Nuevo Leon, Baja California, Mexico, CP. 21705.

[2] Industrias Bioquim, Mexicali, Baja California, Mexico, C.P. 21100

* Correspondence: danielg@uabc.edu.mx

ABSTRACT

Objective: To obtain chitosan oligosaccharides (COS) and evaluate COS uses for the obtention of nanosystem based on silica as vehicle and compare the COS-silica nanosystem with the chitosan (Chi) precursor system as Chitosan-silica nanosystems.

Design/methodology/approach: A combination of hydrolysis chemical and mechanical (microwave assisted) were used to obtain COS with the oxidative action of hydrogen peroxide. Sol-Gel adapted method was used to synthetize silica nanoparticles (SiNPs) from sodium metasilicate and the electrostatic interactions between SiNPs and Chi/COS were used to functionalize the SiNPs surface with Chi/COS.

Results: Nanosystem composed from COS and SiNPs were obtained successful as A COS-SiNPs and C COS-SiNPs with particle size of 139.35 nm and 251.8 nm and zeta potential of 30.40 mV and 34.67 mV respectively with antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Limitations on study/implications: Stabilize the systems compound of chitosan-silica nanoparticles due to the molecular weight of chitosan which loss the stabilized the SiNPs suspension and due the incompatibility of both systems pH.

Findings/conclusions: COS and COS-SiNPs stable systems were obtained with an improvement of the antimicrobial activity of the system in contrast of Chi-SiNPs systems.

Keywords: chitosan, silicon, Sol-Gel. Chemical hydrolysis, mechanical hydrolysis, microwave assisted.

Citation: Salazar-Navarro, A. A., Rivera-Reyna, N. E., & González Mendoza, D. (2023). Synthesis of silica chitosan oligosaccharides nanoparticles *Agro Productividad*. https://doi.org/10.32854/agrop.v16i11.2728

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: June 19, 2023.
Accepted: October 05, 2023.
Published on-line: December 27, 2023.

Agro Productividad, 16(11). November. 2023. pp: 107-114.

INTRODUCTION

Chitosan is considered as the second most abundant polysaccharide followed by cellulose; chitosan is a biodegradable biopolymer derived from chitin by a deacetylation process. Chitin/Chitosan can be obtained from crustaceous exoskeleton, insects, algae, and from the cell wall of some fungus. Chitin is deacetylated and depolymerized to obtain chitosan in alkaline conditions where acetyl groups are delinked from the saccharide chain, thus reducing the affinity of the polysaccharide to stay attached making chitosan more water soluble and less viscous [1]. Chitin and chitosan are compounded from the same saccharide monomers whit the main difference in the proportion of the units in the polysaccharide structure, chitin is mostly compound with N-acetyl-D-glucosamine (GlcNAc) with a portion of D-glucosamine units (GlcN) and the saccharide units are joined by a β 1-4 glycosidic bond. In contrast, chitosan is mainly compound by GlcN units with a about 10% content of GlcNAc, the ration between GlcN and GlcNAc units defines the deacetylated grade of chitosan [2-4].

Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2024.049612

REVIEW

Coffee Leaf Rust (*Hemileia vastatrix*) Disease in Coffee Plants and Perspectives by the Disease Control

Alexis Salazar-Navarro[1], Victor Ruiz-Valdiviezo[2], Jose Joya-Dávila[3] and Daniel Gonzalez-Mendoza[1,*]

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[2]Laboratorio de Biología Molecular del Instituto Tecnológico Nacional de Tuxtla Gutierrez (TecNM), Tuxtla Gutierrez, Chiapas, 29050, México

[3]Facultad de Ciencias Agronómicas Campus V. Universidad Autónoma de Chiapas (UNACH), Villaflores, Chiapas, 30470, México

[*]Corresponding Author: Daniel Gonzalez-Mendoza. Email: danielg@uabc.edu.mx

Received: 12 January 2024 Accepted: 02 April 2024 Published: 28 May 2024

ABSTRACT

Coffee Leaf Rust (CLR) is caused by *Hemileia vastatrix* in *Coffea* spp. It is one of the most dangerous phytopathogens for coffee plantations in terms of coffee productivity and coffee cup quality. In this review, we resume the problem of CLR in Mexico and the pathogenesis of *H. vastatrix*. The review abord plant-pathogen interactions which lead a compatible or incompatible interactions and result in CLR disease or resistance, respectively. The review abord *Coffea* spp. defense response pathways involved in *H. vastatrix* pathogenicity. Additionally, current measures to control *H. vastatrix* proliferation and germination were aborded focused on phytosanitary actions, and biological and chemical control. Finally, new trendlines to reduce the impact of CLR as nanoparticles and nanotechnology were analyzed.

KEYWORDS

Coffee leaf rust; *Coffea arabica*; pathogenesis; nanoparticles; biological control

Nomenclature

AgNPs	Silver nanoparticles
CLR	Coffee Leaf Rust
CBB	Coffee Berry Borer
CeNPs	Cerium nanoparticles
CeONPs	Cerium oxide nanoparticles
CLM	Coffee Leaf Miner
CMCS	Carboxymethyl chitosan
CSB	Coffee Stem Borers
CS	Chitosan
CuNPs	Copper nanoparticles
CuO-NPs	Copper oxide nanoparticles
Cu/Zn-NPs	Bimetallic copper/zinc nanoparticles
CWSB	Coffee White Stem Borer

eISSN: 1984-3909
Coffee Science, e192242, 2024

Doi: https://doi.org/10.25186/.v19i.2242

REVIEW
Potential of ectomycorrhizal and endomycorrhizal fungi in *Coffea* spp. plantations

Alexis Salazar-Navarro[1], Victor Ruiz-Valdiviezo[2], Jose Joya-Davila[1], Daniel Gonzalez-Mendoza[1]

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Received in June 17, 2024 and approved in October 10, 2024

ABSTRACT

The agroecosystem where coffee plantations are located can determine the longevity, health, and quality of coffee beans and plants. In this context, it is important to consider ectomycorrhizal and endomycorrhizal fungi to properly manage coffee plantations, especially in naturally shaded systems where several organisms interact with coffee plants. The microorganisms in the rhizosphere are determinant to achieve the proper nutrition in coffee plants. Endomycorrhizal or arbuscular mycorrhizal can complete a mutualistic symbiotic interaction directly with coffee plants, preparing the plants for future attacks of pathogens by maintaining their defense mechanisms alert. To date, ectomycorrhizal fungus has not been reported to establish a symbiotic interaction directly with coffee plants. Still, it is present in naturally shaded coffee plantations, probably due to their interaction with surrounding trees. Ectomycorrhizal fungi can also improve and alert the defense mechanism of plants, and Both ecto- and endomycorrhizal interactions can enhance the uptake of micro and macronutrients in coffee plants and improve the soil organic matter uptake, which can positively impact the coffee quality. This review establishes the importance of deepening research on mycorrhizal interaction with coffee plants.

Key words: Inorganic phosphorous deficiency; Myc factors; mycorrhizal-induced resistance; symbiotic interactions; systemic acquired resistance.

1 INTRODUCTION

The *Coffea* genus belongs to the *Rubiaceae* family and is cultivated in 80 countries. Within *Rubiaceae*, the *Coffea* genus has major economic importance. It comprises 124 species, but only *Coffea arabica*, *Coffea canephora*, *Coffea liberica*, and *Coffea excelsa* have the economic potential to be cultivated (Ferreira et al., 2021). Among these, *C. arabica* and *C. canephora* are the major economically important species responsible for 60-70% and 30-40% of global production, respectively. *C. canephora* is also known as robusta coffee due to its vigor, hardness, resistance to pathogen attacks, and higher caffeine content in comparison with *C. arabica* (Davis et al., 2006; Rojo Jiménez, 2014; Romero; Camili, 2019).

Coffee is native to the forest and tropical zones of Africa (Ethiopia, Sudan, Kenya, Guinea, and Mozambique) and was spread to Arabia, Yemen, and Egypt (Century XV). *C. arabica* is native to Ethiopia and Sudan and results from a natural hybridization between *Coffea eugenioides* and *C. canephora* (Batista et al., 2015; Rojo Jiménez, 2014; Romero; Camili, 2019; Scalabrin et al., 2020). These species have different contents of the alkaloid caffeine, one of the main bitter components in coffee cups: *C. eugenioides* has the lowest caffeine content, followed by *C. arabica* and *C. canephora* (Münchow et al., 2020; Ne Perrois et al., 2015; Vitzthum, 1999). The most common varieties are derived from the ancestral species in Yemen, for example, *C. arabica var. Typica* and *C. arabica var. Borbon* (Batista et al., 2015; Ne Perrois et al., 2015; Romero et al., 2010). Some *Coffea* species result from natural hybridization between *C. arabica* and *C. canephora* and are known as Hybrids of Timor (HDT). HTD has resistance genes for Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix*. Some *C. arabica* subspecies were developed by breeding, e.g. the hybrids of Catimor originated from crosses between *C. arabica* var. Caturra and HDT. Catimor hybrids were developed to produce *C. arabica* varieties resistant to CLR (Salazar-Navarro et al., 2024; Salojärvi et al., 2024).

Depending on the coffee fermentation process and roast profile, *C. arabica* beans have a variety of flavors overlapping with tea profiles (e.g., green tea, black tea, and jasmine tea), citrus fruits (the presence of citric acid and malic acid), tartaric fruits, and even acetic profiles due to the fragmentation of carbohydrates into aliphatic acids (Münchow et al., 2020; Sunarharum et al., 2014).

C. canephora is an autochthonous tree species from Uganda and South Sudan that can grow up to 10 m in height. Compared with other species, C. canephora has smaller cherries and beans with spherical morphology, whereas *C. arabica* beans have oval morphology. After the roasting process, *C. canephora* generally presents a bitter cup profile due to its higher caffeine and chlorogenic acid content (Nair, 2010; Ne Perrois et al., 2015; Rojo Jiménez, 2014; Romero; Camili, 2019).

Plants are colonized by diverse microorganisms both above- (phyllosphere, including fruits, flowers, leaves, and stems) and belowground (rhizosphere) (Li et al., 2021). Plants can recruit beneficial microorganisms by photosynthetic exudates, e.g. Plant Growth Promoting Rhizobacteria (PGPB)

Phyton-
International Journal of
Experimental Botany

Tech Science Press

Doi:10.32604/phyton.2025.062871 Published Online: 19 March 2025

ARTICLE

Coffea arabica var. Borbon Biochemical Response to Chitosan Oligosaccharides Foliar Exposure

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Received: 30 December 2024; Accepted: 20 February 2025

ABSTRACT: The biochemical response of *Coffea arabica* var. Borbon to chitosan and chitosan oligosaccharides (COS) was evaluated in one-year-old plants under greenhouse conditions. COS solutions were synthesized through chemical and physical hydrolysis using acetic acid, hydrogen peroxide, and microwave irradiation. The obtained COS had an average molecular weight (Mw) of 3549.90 ± 0.33 Daltons (Da), a deacetylation degree (DD) of 76.64 ± 1.12%, and a polymerization degree (PD) of 18.91 ± 0.0018. Solutions of chitosan and COS were applied to *C. arabica* var. Borbon at concentrations of 0.25, 0.5, and 1 wt%. The experimental design was conducted using a completely randomized design with four replications. The biochemical responses assessed included soluble protein content, phenylalanine ammonia-lyase (PAL), chitinase, β-1,3-glucanase, peroxidase, catalase, and chlorophyll fluorescence. The application of COS demonstrated significant differences ($\alpha = 0.05$) in protein concentration, with the activity of β-1,3-glucanase, chitinase, and catalase being 1.5, 7.5, and 3.9 times higher, respectively, while showing similar behavior to chitosan in PAL activity, both up to 4.4 times higher than the distilled water control and lower than chitosan in peroxidase activity. Treatments with chitosan yielded a higher photochemical efficiency of Photosystem II (PSII). The application of COS suggests a viable foliar alternative to active plant defense mechanisms without the risk of phytotoxicity.

KEYWORDS: Enzymatic activity; PAL; β-1,3-glucanase; chitinase; chlorophyll fluorescence; photosystem II

1 Introduction

The global coffee trade is one of the most important commodity markets worldwide [1]. In 2023, coffee production reached 171.4 million bags (60 kg), with Brazil as the leading coffee-producing country, followed by Vietnam. Mexico ranked 10th in global coffee production [2], with 81%–82% of its output concentrated in the states of Chiapas, Veracruz, and Puebla [3]. In 2023, approximately 87% of Mexico's total coffee production was *Coffea arabica*, while the remaining percentage was *Coffea canephora*, commonly known as arabica and robusta, respectively [2]. *C. arabica* generates higher income in Mexico due to its cup qualities, but it also presents production challenges due to its susceptibility to pests and diseases [4]. For instance, Coffee Leaf Rust (CLR), caused by *Hemileia vastatrix*, an obligate biotrophic fungus, and Coffee Berry Disease (CBD), caused by *Colletotrichum kahawae* Waller & Bridge [5], are among the most threatening diseases for coffee plantations. *H. vastatrix* infections in *C. arabica* plantations can cause yield losses of up to

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Article

Impact of Temperature on the Bioactive Compound Content of Aqueous Extracts of *Humulus lupulus* L. with Different Alpha and Beta Acid Content: A New Potential Antifungal Alternative

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Abstract: Hops contain a wide variety of polyphenolic compounds with diverse antimicrobial properties. This study aimed to evaluate the impact of temperature on the bioactive components of samples of aqueous extracts of hops with different characteristics. A central compound rotating design model was used in order to obtain optimal conditions of temperature and extract concentration to inhibit *Fusarium oxysporum* and *Alternaria solani*. At intermediate temperatures according to the design of experiments, significant effects on antifungal activity were observed. The optimal conditions with antifungal activity were at a concentration of 160 mg/mL and a temperature of 65 °C to obtain mycelial diameters ≤ 25 mm. The bioactive compounds were shown in the FT-IR spectrum after each heat treatment of both samples; significant changes were observed in the bands between 2786 to 3600 cm^{-1} and 1022 to 1729 cm^{-1}. The content of total phenols and flavonoids showed a concentration increase of 4.54 to 6.24 mg GAE/g and 6.21 to 8.12 mg QE/g from an initial evaluation temperature of 25 °C to 57.5 °C, respectively, benefited by the heating temperature, enhancing antifungal activity. However, when increasing the temperature ≥90 °C, a tendency to decrease the concentration of bioactive compounds was observed, probably due to their denaturation due to the effect of temperature and exposure time, being non-thermolabile compounds at high temperatures. These aqueous extracts are an alternative to effective natural antifungals.

Keywords: *Humulus lupulus* L.; FT-IR; antifungal activity; aqueous extract; optimization

Citation: Basilio-Cortes, U.A.; Tzintzun-Camacho, O.; Grimaldo-Juárez, O.; Durán-Hernández, D.; Suarez-Vargas, A.; Durán, C.C.; Salazar-Navarro, A.; González-Anguiano, L.A.; González-Mendoza, D. Impact of Temperature on the Bioactive Compound Content of Aqueous Extracts of *Humulus lupulus* L. with Different Alpha and Beta Acid Content: A New Potential Antifungal Alternative. *Microbiol. Res.* **2023**, *14*, 205–217. https://doi.org/10.3390/microbiolres14010017

Academic Editor: Premila Achar

Received: 16 January 2023
Revised: 1 February 2023
Accepted: 4 February 2023
Published: 6 February 2023

1. Introduction

Microorganisms of the genus of filamentous such as *Fusarium oxysporum*, *Rhi-zoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Alternaria brassicicola* among others are phytopathogens worldwide, causing great damage and losses in agricultural production of different varieties of crop plants and even of postharvest fruits and vegetables with blight disease [1–4]. The main prevention used for decades has been the indiscriminate use of chemical fungicides (e.g., benomyl, carbendazim, thiabendazole, and alliete); however, they have caused pathogens to generate resistance in addition to promoting environmental contamination in crops, further potentiating the food insecurity and human health [5–7].

Effective natural compounds with activity to inhibit the development and growth of pathogenic microorganisms against fungi and bacteria are innovative trends for the immediate replacement of potentially toxic and environmentally harmful chemicals. Postharvest fruit conservation aims to extend shelf life and maintain a safe product. Various investigations have reported effective biomolecules from plant extracts due to their low cost and high performance, propitiating this effect due to the content of bioactive compounds and

fermentation

Article

Spectroscopic Analysis of Selenium Nanoparticles Synthesized by *Saccharomyces boulardii* for the Production of Craft Beer

Lourdes González-Salitre [1,2], Luis Guillermo González-Olivares [3,*], Alexis Alejandro Salazar-Navarro [1], David Cervantes-García [1], Dagoberto Durán-Hernández [1], Ricardo Torres-Ramos [1], Mary Triny Beleño-Cabarcas [1] and Ulin Antobelli Basilio-Cortes [1,*]

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Abstract: Selenium is an essential micronutrient which is found in many foods and beverages in low concentrations. Craft beer, one of the most widely consumed fermented beverages globally, presents a strategic opportunity for selenium intake through organic nanoparticles. This study aimed to confirm the presence of selenium nanoparticles in the fermentation process of an ale-style beer using *S. boulardii* yeast selenized with Na_2SeO_3 (74 ppm), through spectroscopic analysis and TEM. The yeast accumulated 5.92 mg/g of dry cell mass, and the beer contained 0.642 mg/g of selenium. UV-VIS detected nanoparticles with a peak at 300 nm and FT-IR at a wavelength of 1398.85 cm^{-1}. The particle size ranged between 74 to 175 nm, with a maximum ζ-potential of −4.2 mV, an electrophoretic mobility of −0.3492 μm × cm Vs^{-1}, and a conductivity of 2.656 mS cm^{-1}. TEM analysis revealed that the nanoparticles exhibited circular/ovoid shapes. The fermentation process, combined with the ingredients used to produce ale-type craft beer, proved to be a feasible method for the biosynthesis of selenium nanoparticles using *S. boulardii*, offering a reliable option for developing and innovating functional craft beers.

Keywords: *S. boulardii*; nanoparticles; selenium; craft beer; spectroscopy

Academic Editor: Giuseppe Italo Francesco Perretti

Received: 27 February 2025
Revised: 7 March 2025
Accepted: 8 March 2025
Published: 13 March 2025

Citation: González-Salitre, L.; González-Olivares, L.G.; Salazar-Navarro, A.A.; Cervantes-García, D.; Durán-Hernández, D.; Torres-Ramos, R.; Beleño-Cabarcas, M.T.; Basilio-Cortes, U.A. Spectroscopic Analysis of Selenium Nanoparticles Synthesized by *Saccharomyces boulardii* for the Production of Craft Beer. *Fermentation* **2025**, *11*, 144. https://doi.org/10.3390/fermentation11030144

1. Introduction

Selenium (Se) is an essential, functional micronutrient that is crucial to human health. According to the National Institute of Health (NIH), the recommended daily intake of Se for adults is 55 μg/day [1]. S exists in organic forms, such as selenomethionine (SeMet), selenocysteine (SeCys), selenocysteine (SeCys2), and methylselenocysteine (MeSeCys), as well as inorganic forms like SeVI and SeIV. Both forms exert various bioactive effects, including antioxidant, anticancer, fertility, reproductive, metabolic, and immune system functions [2–6]. Insufficient Se intake has been linked to chronic degenerative diseases. Se is now recognized as an essential trace element for human health, and extreme deficiencies have been observed worldwide, affecting individuals of all ages. Therefore, it is vital to address Se deficiency by supplementing with selenium-enriched foods or yeast biomasses.

plants

Article

Variability in Anthocyanin Expression in Native Maize: Purple *Totomoxtle* as a Phenotypic Trait of Agroecological Value

José Gregorio Joya-Dávila [1,*], Federico Antonio Gutiérrez-Miceli [2,*], Leslie Alondra Serrano-Gómez [2], Alexis Salazar-Navarro [1], Daniel González-Mendoza [1], Olivia Tzintzun-Camacho [1], Ana Martín Santos-Espinoza [2], Grisel Sánchez-Grajalez [3,*], Eraclio Gómez-Padilla [3] and Jaime Llaven-Martínez [3]

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Abstract

Purple *totomoxtle* (maize husk) in native maize represents a phenotypic trait of cultural and agronomic significance within traditional Mesoamerican agroecosystems. This study evaluated the phenotypic expression of anthocyanins in vegetative and reproductive tissues of ten native maize genotypes, including inter-parental crosses derived from both pigmented and non-pigmented lines. Field trials were conducted under rainfed conditions in Chiapas, Mexico. Visual and quantitative assessments included pigmentation intensity, chlorophyll and carotenoid content, ear traits and appearance, grain health, and yield performance. Genotypes exhibiting the purple phenotype showed consistent pigment accumulation in stems, nodes, leaf sheaths, tassels, and bracts (totomoxtle), with statistically significant differences compared to non-pigmented controls. Anthocyanin content in totomoxtle increased by 30% during late developmental stages, whereas chlorophyll and carotenoid levels peaked during early vegetative growth. Pigmented materials displayed healthier grain, enhanced ear appearance, and higher yields, with the JCTM × LLMJ cross reaching 6.60 t ha^{-1}. These findings highlight the functional value of purple totomoxtle and its potential in agroecological programs aimed at resilience, genetic conservation, and integral resource utilization, providing useful criteria such as stable pigment expression and superior yield to guide sustainable reproduction strategies.

Keywords: native maize landraces; pigment-based selection; anthocyanin accumulation; nutritional metabolites; *totomoxtle* pigmentation; phenotypic trait conservation

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Academic Editors: Farhat Abbas, Yiwei Zhou and Yanguo Ke

Received: 16 July 2025
Revised: 6 August 2025
Accepted: 9 August 2025
Published: 12 August 2025

Citation: Joya-Dávila, J.G.; Gutiérrez-Miceli, F.A.; Serrano-Gómez, L.A.; Salazar-Navarro, A.; González-Mendoza, D.; Tzintzun-Camacho, O.; Santos-Espinoza, A.M.; Sánchez-Grajalez, G.; Gómez-Padilla, E.; Llaven-Martínez, J. Variability in Anthocyanin Expression in Native Maize: Purple *Totomoxtle* as a Phenotypic Trait of Agroecological Value. *Plants* **2025**, *14*, 2511. https://doi.org/10.3390/plants14162511

1. Introduction

Maize (*Zea mays* L.), a member of the Poaceae family, is a Mesoamerican crop domesticated over 9000 years ago, with its center of origin located in regions that now comprise Mexico and Guatemala [1,2]. It is one of the fundamental pillars of global agri-food systems, owing to its nutritional value and its versatility as a raw material in the food, energy, and pharmaceutical industries. In Mexico, national average yields are estimated at 3.9 tons per