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CENTRO UNIVERSITARIO DE CIENCIAS BIOLÓGICAS Y AGROPECUARIAS

DIVISIÓN DE CIENCIAS BIOLÓGICAS Y AMBIENTALES
DEPARTAMENTO DE BOTÁNICA Y ZOOLOGÍA

Mecanismos físico-químicos (estructuras histológicas y metabolitos secundarios) de resistencia en cultivares de *Opuntia ficus-indica* (L.) Mill. a *Dactylopius coccus* Costa

Tesis Presentada por el Sustentante:

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Como Requisito Parcial para Obtener el Grado de:

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DECLARATORIA DE ORIGINALIDAD

A quien corresponda:

Por este conducto el abajo firmante, Yemane Kahsay Berhe, autor del Trabajo Recepcíonal (Tesis) titulada: “Mecanismos físico-químicos (estructuras histológicas y metabolitos secundarios) de resistencia en cultivares de *Opuntia ficus-indica* a *Dactylopius coccus*”, que el contenido del mismo constituye un documento inédito y original por lo que cumple con los términos de originalidad a los que se hace mención en el Artículo 73 del Reglamento General de Posgrado y Articulo 14 fracción I del Reglamento General de Titulación de la Universidad de Guadalajara.

ATENTAMENTE

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Por este medio nos permitimos informar a ustedes que habiendo revisado el trabajo de Tesis de Doctorado, titulado "**Mecanismos físico-químicos (estructuras histológicas y metabolitos secundarios) de resistencia en cultivares de *Opuntia ficus-indica* a *Dactylopius coccus*",** que realizó el sustentante **Yemane Kahsay Berhe** con número de código **219925218**, este comité abajo firmante consideramos que el trabajo ha quedado debidamente concluido, por lo que ponemos a su consideración el escrito final para autorizar su envío a los lectores/sinodales y una vez aprobado enviar a impresión y programar la fecha de examen de titulación respectivo.

Sin otro particular agradecemos de antemano la atención que se sirva brindar a la presente y aprovechamos la ocasión para enviarle un cordial saludo.

Atentamente
Las Agujas, Nextipac, Zapopan, Jalisco, 07 de junio de 2023

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PRESENTE

Una vez que fue revisada y corregida la tesis de doctorado titulada: "Mecanismos físico-químicos (estructuras histológicas y metabolitos secundarios) de resistencia en cultivares de *Opuntia ficus-indica* a *Dactylopius coccus*" por el M.C. Yemane Kahsay Berhe, tesis presentada como requisito parcial para obtener el grado de Doctor en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas, consideramos que cuenta ya con la calidad académica necesaria para defensa de grado.

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DEDICATORIAS

Dedicado a mi padre, a quien extrañé durante el período de estudio.

Dedicado a las víctimas de Tigray de la guerra genocida, que perdieron la vida y se deshumanizaron.

Dedicado a los héroes tigriños quienes defendieron a su tierra durante la guerra genocida 2020-2023.

Physicochemical mechanisms (histological structures and secondary metabolites) of resistance in *Opuntia ficus-indica* (L.) Mill. cultivars to *Dactylopius coccus* Costa

Yemane Kahsay Berhe

ABSTRACT

This thesis includes six chapters. The introductory chapter describes the importance of *Opuntia ficus-indica* and the Dactylopidae pests, a brief of *Dactylopius coccus*, the mechanism of resistance in *Opuntia*, and the propagation and forage performance of pest resistance *Opuntia* cultivars. The second chapter includes the result of the resistance evaluation of cultivars and genotypes. In the third chapter physical mechanism of insect resistance in *Opuntia* was reported. Cladode characteristics, especially oxalate number and epidermis thickness were confirmed to be associated with resistance. Phytochemistry and secondary metabolites in relation to pest resistance were assessed in chapter four. Protein, flavonoids, peroxide, and total polyphenols contents were examined in relation to *D. coccus* resistance. Resistance-related secondary metabolites were also profiled with DLI-ESI MS. The fifth chapter has a report on cv. 'Rojo Pelón' micropropagation protocol adaptation results. The cultivar was able to be cultivated *in vitro* and the plantlets had *ex vitro* establishment. Nutritional analysis was reported, and the resistant cultivar was confirmed to have acceptable nutritional content compared to the susceptible cultivars. The sixth and final chapter is the conclusion of the thesis that summarizes the major findings from all chapters.

Mecanismos físico-químicos (estructuras histológicas y metabolitos secundarios) de resistencia en cultivares de *Opuntia ficus-indica* (L.) Mill. a *Dactylopius coccus* Costa

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RESUMEN

Esta tesis incluye seis capítulos. El primer capítulo introductorio describe la importancia de *Opuntia ficus-indica* y las plagas Dactylopiidae, un resumen de *Dactylopius coccus*, el mecanismo de resistencia en *Opuntia*, y la propagación y desempeño forrajero de los cultivares de *Opuntia* resistentes a plagas. El segundo capítulo incluye el resultado de la evaluación de resistencia de cultivares y genotipos. El capítulo demostrado el mecanismo de resistencia de los insectos en *Opuntia*, se confirmó que las características de los cladodios, especialmente el número de oxalatos y el espesor de la epidermis, están asociados con la resistencia. La fitoquímica y los metabolitos secundarios en relación con la resistencia a las plagas se evaluaron en el capítulo cuatro. Se examinaron los contenidos de proteína, flavonoides, peróxido y polifenoles totales en relación con la resistencia a *D. coccus*. Los metabolitos secundarios relacionados con la resistencia también se perfilaron con DLI-ESI MS. El quinto capítulo tiene un informe sobre la micropropagación de cv. 'Rojo Pelón'; resultados de la adaptación del protocolo de micropropagación de 'Rojo Peló; el cual se pudo propagar *in vitro* y las plántulas se establecieron *ex vitro*. Además del análisis nutricional del cultivar resistente que presentó un contenido nutricional aceptable en comparación con los cultivares susceptibles. El sexto y último capítulo es la conclusión de la tesis que resume los principales hallazgos de todos los capítulos.

TABLA DE CONTENIDOS

CAPÍTULO 1. INTRODUCCIÓN GENERAL

1.1. Domesticación y distribución de *Opuntia ficus-indica*

Opuntia es el género más diverso de las cactáceas, originario y ampliamente distribuido en Mesoamérica (Christenhusz y Byng, 2016). La reproducción sexual parece haber contribuido a su éxito ecológico y evolutivo (Reyes-Agüero et al., 2005). Algunas especies, como *O. ficus-indica, O. robusta* (sin espinas) y *O. undulata* se supone que son el resultado de la selección humano, ya que sólo se encuentran en cercas creadas por el hombre (Britton y Rose, 1919). Se plantea la hipótesis de que *O. ficus-indica* consiste en un grupo de múltiples clones no relacionados, derivados de diferentes especies parentales y seleccionados por diferentes características agronómicas (Kiesling et al., 2017). *O. ficus-indica, O. albicarpa y O. megacantha* posiblemente tengan un ancestro común (Valadez-Moctezuma et al., 2014). Reyes-Agüero et al. (2005) explicaron que *O. ficus-indica* es una especie diferente a *O. amyclae, O. megacantha y O. streptacantha*. *O. ficus-indica* tiene estrecha relación con *O. albicarpa, O. fusicaulis, O. amyclaea, O. elizondoana, O. megacantha, O. joconostle, O. undulata y O. vulgaris* (Mazri, 2018). La especie puede considerarse una especie domesticada de *O. megantha* (Labra et al., 2003; Reyes-Agüero et al., 2005), ya que es cultivada desde hace mucho tiempo al ser importante en la agricultura y alimentación humana (Griffith, 2004; Del Socorro Santos Díaz et al., 2017; Taylor, 1994). Las especies del género *Opuntia* pierden su capacidad de supervivencia que tienen en estado silvestre, porque la domesticación favorece la nutrición y el rendimiento (Astello-García et al., 2015), y su morfología y química se modifican sin un patrón identificable (López-Palacios y Peña-Valdivia, 2020; Ferrero et al., 2020; Follett, 2017).

Los antiguos mexicanos seleccionaron a *O. ficus-indica* como fuente de frutas y cladodios tiernos, a partir de una planta silvestre para los jardines familiares para asegurar el consumo doméstico (Kiesling et al., 1998; Griffith, 2004). La urbanización empujó a los agricultores a convertir el nopal en un cultivo formal en menos de seis décadas, adaptable a diferentes entornos y sistemas de producción (Gallegos-Vázquez et al., 2009; Salinas, 2018). Esta planta fue domesticado desde hace 9000 años con cambios de selección prolongada, cruces y retrocruzamientos (Griffith, 2004; López-Palacios y Peña-Valdivia, 2020; Kiesling, 1998). Se desarrollaron plantas vigorosas con poliploidía (Kiesling, 1998), además debido a la dispersión de semillas, hay mucha diversidad intraespecífica en otros países fuera de su origen.

Después del descubrimiento de América, *O. ficus-indica* fue introducida en España (1500) y, más tarde, en toda la cuenca mediterránea por marineros, Italia (1526), luego en el siglo XVIII a otros países (Kiesling, 1998). Ahora se cultiva en alrededor de 25 países principalmente por su fruta, nopal (verdura) y forraje (Ochoa y Barbera, 2017).

El nopal, también conocido como beles en Tigriño, se introdujo en la región de Tigray, Etiopía entre 1848 y 1920 (Neumann, 1997), se encuentra ampliamente distribuido en las regiones áridas y semiáridas del país; especialmente en las zonas este y sur de Tigray (Lemma et al., 2010). En las últimas décadas ha aumentado el interés por el nopal como alimento y forraje debido a su resistencia a la sequía, alto rendimiento de biomasa, alta palatabilidad y tolerancia a la salinidad (Barbera et al., 1992). Stintzing y Carle (2005) caracterizaron al nopal como una “planta milagrosa, dromedario del mundo vegetal y banco de la vida”, ya que puede contribuir al sustento de las poblaciones rurales en zonas secas. Por eso, nopal puede asumir una mayor importancia agrícola en las zonas secas, ya que una mayor porción de la tierra está destinada a volverse árida o semiárida debido al cambio climático (Gebretsadik et al., 2013). Casi todas las comunidades rurales de la zona oriental y sur de Tigray, y algunas partes de Tigray central utilizan nopal como alimento básico durante unos cuatro meses y como cultivo comercial, lo que cubre 360 000 ha (Brutsch, 1997). Otro beneficio del nopal es también su capacidad para absorber y retener el exceso de CO^2 de la atmósfera (Mazri, 2018).

Además, el nopal es una parte importante del patrimonio cultural y un recurso alimentario para la gente de la región de Tigray (Tegegne et al., 2005). El nopal se ha transformado en una parte integral de la cultura y la economía de Tigray y se utiliza de muchas formas, aunque no como verdura (Brutsch, 1997). En Tigray, esta especie se considera el 'Puente de la vida' porque los tallos y los frutos almacenan grandes cantidades de agua y proporcionan tanto alimento para el ganado en épocas de sequía como alimento para los pastores, contribuyendo así a su supervivencia (SAERT, 1994). Es fuente de ingresos durante el verano (Mondragón-Jacobo y Tegegne, 2006). Para mejorar la gestión de los huertos y el rendimiento de la fruta, la Oficina de Agricultura y Desarrollo Rural de Tigray estableció huertos de beles mejorados, lo que implica que beles es un cultivo de frutas y forraje muy importante (Mondragón-Jacobo y Tegegne, 2006).

También se integró en la sostenibilidad agricultura y rehabilitación ambiental en Tigray (SAERT) que se desarrolló en 1994 (SAERT, 1994). Un estudio de caso realizado en un pueblo llamado Kihen del distrito de Kilteawlaelo muestra que los agricultores de Tigray generan alrededor del 25% de sus ingresos anuales a través de las actividades relacionadas con los nopales (De Bac, 2010). El nopal es significativo para la generación de ingresos y como alimento para todo tipo de animales en áridas y semiáridas (Arba, 2022).

1.2 *Dactylopius coccus* Costa

Dactylopius coccus es la especie cultivada de cochinilla (Portillo y Vigueras, 2006; Van Dam y May, 2012), que produce el tinte carmín y está siendo utilizada como producto comercial en diferentes países (Piña, 1979). Este colorante tiene varios usos especialmente, como aditivo para alimentos, medicamentos, cosméticos y textiles (fibras) (Torres-Ponce et al., 2015; Arroyo-Figueroa et al., 2016; y este colorante es preferido por su alta estabilidad a la luz y temperatura, además de ser inocuo para la salud humana, por ser de origen natural (Müller-Maatsch y Gras, 2016). *D. coccus* es predominantemente dependiente de *O. ficus-indica* (Kiesling, 1998). Los insectos cochinilla son parásitos obligados de los nopales (principalmente *Opuntia* spp.), con especies/biotipos individuales de *Dactylopius* que prefieren diferentes especies de *Opuntia* como hospederos (Volchansky et al., 1999; Hoffmann et al., 2002; Campana et al., 2015; Portillo y Vigueras, 2006). *D. coccus* se cultiva principalmente en *O. ficus-indica* (Campana et al., 2015).

En etapa de ninfa (también llamada etapa de rastreo) es que la cochinilla se dispersa. Estos individuos establecen sitios de alimentación en el nuevo hospedero y producen una nueva generación de cochinillas (Nejad y Nejad, 2013). Los individuos en esta etapa se trasladan a un lugar de alimentación, generalmente al borde de los cladodios del nopal, donde comienzan a producir largos filamentos de cera. Estos filamentos cerosos les permiten un mayor arrastre por el viento, logrando así con mayor facilidad el traslado a un nuevo huésped. Son estos individuos los que establecen sitios de alimentación en el nuevo huésped y producen una nueva generación de cochinillas (Eisner et al., 1994).

La hembra es un insecto de metamorfosis incompleta, el ciclo de vida de la hembra tiene cuatro estados: huevo, primer y segundo estadios de ninfa y adulto. Mientras que el macho es un insecto de metamorfosis completa, su ciclo de vida tiene seis estados: huevo, estadio ninfal, prepupa (ya en capullo), pupas y adulto (Roque-Rodríguez, 2022; Zhang, 2017).

1.3 Problema de *Dactylopius coccus* Costa como plaga

En Tigray, Etiopia, la introducción intencional de cochinilla carmín (*D. coccus*) en 2011 para la producción de ácido carmínico disminuyó severamente la producción del cultivo, este insecto se diseminó agresivamente y destruyó miles de hectáreas de nopal en pocos años; su escala de invasión ha sido tan severa que hay áreas donde las plantas infestadas ya no producen frutos y tampoco sirven como fuente de alimento para el ganado. En algunas localidades, la infestación ha provocado una erosión severa del suelo en áreas que solían estar cubiertas por estas plantas. Como resultado de la gravedad del daño a las plantas, el insecto cochinilla ha sido etiquetado como una "amenaza para la producción de tunas en Tigray" porque se volvió invasivo y dañó muchas plantaciones (Belay, 2015; Shushay, 2014, Berhe et al., 2020).

La invasión de enormes áreas potenciales por la plaga de la cochinilla carmín (*D. coccus*) que fue introducida para beneficio adicional (Belay, 2015) ha tenido un impacto negativo directo en los aspectos económicos (alimentos, forrajes y generación de ingresos), sociales y ambientales (conservación del suelo y el agua, mitigación del cambio climático) aspecto de la región (Ochoa y Barbera, 2017); porque su contribución al ingreso del hogar representa hasta 25% (Taddele y Workneh, 2010).

La infestación por cochinilla aumentó de 3.8 ha a 75 000 ha en poblaciones de plantas tanto silvestres como en traspatio, lo que indica que el insecto se está expandiendo a un ritmo alarmante (Berhe y Siyum, 2022). Las condiciones climáticas favorables, como un largo período seco y la abundancia de vegetación de nopal en zonas silvestres y densas, la ausencia de enemigos naturales y una cuarentena y control locales efectivos de plagas, agravaron la colonización (Belay, 2015), trajo pérdidas económicas y sociales y degradación de los recursos naturales. La pérdida económica causada por la plaga de insectos implica reducción de ingresos, número de cabezas de ganado y del rendimiento de los cultivos debido al ataque de animales grandes que habían sido cercados con plantas de nopaly falta de bueyes para arar, aumento de la dependencia de la ayuda alimentaria, fuerte migración de jóvenes y inestabilidad social creada. Como el nopal tiene un gran rol en la conservación del suelo, debido a la infestación de la plaga de insectos, la degradación del suelo se volvió devastadora, no sólo perdiendo el suelo, sino que la inundación creada en las montañas también dañó las tierras de cultivo. Las áreas montañosas se transforman en una tierra desnuda que expone el área a la lluvia directa e inundaciones (Fitiwy et al., 2016; Berhe et al., 2020; Abay, 2018; Hailu, 2020).

1.4 Cultivares de *Opuntia ficus-indica* resistentes a *Dactylopius coccus*

La utilización de variedades de cultivos resistentes a plagas es importante para la economía, el medio ambiente y la ecología. Porque, la reducción de la pérdida de cultivos debido a los insectos y la minimización del costo de los insecticidas, disponibilidad, las semillas de variedades resistentes a insectos son un poco más caras que el costo de las variedades susceptibles o, en ocasiones, pueden ser iguales (Teetes, 1996). Más importante aún, los agricultores no requieren habilidades especiales o técnicas de aplicación (Sharma y Ortiz, 2002). También ayuda a reducir el uso de productos químicos y el riesgo indirecto y relacionado de contaminación ambiental y la salud humana (Munhame et al., 2021; Reinert et al., 2003; Vigueras y Portillo, 2014; El Finti et al., 2022; Berhe et al., 2022). Tovar et al. (2005) informaron que el rendimiento de *D. coccus* obtenido para la var. Villanueva son superiores a otras dos variedades probadas en México.

1.5 Mecanismo de resistencia de las plantas a insectos

Los insectos y las plantas exhiben todo tipo de relaciones descritas en la literatura ecológica, desde el mutualismo hasta la interacción antagónica. Ambos han habitado juntos durante al menos 400 millones de años y, desde entonces, los insectos han buscado sustento en las plantas verdes y las plantas han desarrollado formas de protegerse. Cuando las plantas con flores evolucionaron hace unos 140 millones de años, los insectos se volvieron importantes en la polinización y pueden haber contribuido al éxito de este nuevo grupo de plantas. Todos albergan insectos herbívoros y la mayoría depende de los insectos para transportar el polen de una flor a otra. Muchas de las complejidades de las interacciones de insectos y plantas han sido influenciadas en su evolución por otros organismos, como reptiles y mamíferos herbívoros, que pueden haber proporcionado parte de la presión de selección para los mecanismos de defensa de las plantas (Bernays, 1992). Cada planta interactúa con los insectos de manera diferente; los insectos pueden actuar como protección, dispersores o fertilizantes para las plantas, mientras que las plantas pueden ser un recurso de alimento/energía o un lugar de anidación para los insectos. Las interacciones planta-insecto se consideran clásicamente como mutualistas, antagónicas o comensalismo (Calatayud et al., 2018). Las interacciones microbio-planta-insecto también tienen una diversidad similar (Noman et al., 2020). La teoría evolutiva sugiere que una carrera armamentista entre plantas y herbívoros produce una mayor diversidad de metabolitos especializados en plantas y la teoría del mosaico geográfico de la coevolución predice que las interacciones coevolutivas varían según las escalas geográficas. La carrera armamentista entre las plantas hospederas y los herbívoros es una fuerza impulsora de la coevolución (Ernst et al., 2019).

Las interacciones planta-insecto se desarrollan en un escenario que es mucho más grande que la propia planta. Las plantas tienen innumerables formas de defenderse de sus atacantes, incluida la producción de sustancias químicas defensivas. La producción constitutivamente alta de defensas costosas sólo podría ser beneficiosa para una planta si la presión de los herbívoros es un factor ambiental predecible. Los entornos impredecibles seleccionaron plantas que pueden producir una defensa sólo cuando sea necesario, en presencia de herbívoros. Además, las plantas producen químicos orgánicos volátiles (COV) en respuesta al daño de los herbívoros (Gómez Montenegro, 2023). Estos pueden funcionar como señales para organismos capaces de recibir y responder a ramos de olores modificados, y para atracción de enemigos naturales de insectos como parasitoides y/o depredadores hacia la planta dañada (Kessler, 2006).

Los insectos reconocen eficazmente las plantas huésped que producen metabolitos secundarios específicos a través de sus quimiorreceptores finamente sintonizados. En un proceso similar, los insectos rechazan las plantas dañinas o no hospedantes. La adaptación del huésped y la especiación en insectos están estrechamente relacionadas con tales mecanismos quimio sensoriales: interacciones entre ligandos y quimiorreceptores específicos. La importancia química y ecológica de los metabolitos secundarios de las plantas involucrados en la vida de los insectos como; señales de búsqueda de huéspedes, barreras químicas, alelo químicos para la defensa, para la comunicación sexual y en asociaciones mutualistas como la polinización (Nishida, 2014). Los insectos participan en procesos clave para el bosque y brindan “servicios gratuitos” a los cultivos (e. g., polinización, control de plagas) (McGregor, 1976).

Se pueden distinguir dos amplias categorías de defensas de las plantas: (a) siempre presentes o constitutivas y (b) inducibles, que pueden ser provocadas específicamente por ciertos agresores. Se establece una defensa inducible localmente en el lugar de la infestación, así como sistemáticamente en toda la planta. Cada uno puede ser directo o indirecto, químico o mecánico (características morfológicas como espinas, espinas o altos niveles de lignificación). Las defensas directas actúan por sí solas contra el agresor y las defensas indirectas actúan a través de la atracción de organismos de un nivel trófico adicional, por ejemplo, de enemigos de los herbívoros atacantes (Mithöfer y Boland, 2012). Las defensas indirectas son la producción de volátiles y la secreción de néctar extra floral que median en las interacciones de las plantas con los enemigos naturales de las plagas de insectos (Tibebu, 2018; Mithöfer y Boland, 2012; Gómez Montenegro, 2023). Ambos mecanismos de defensa (directos e indirectos) pueden estar presentes de forma constitutiva o ser inducidos después del daño causado por los insectos (Chaudhary et al., 2018). La respuesta en las plantas es importante del control de plagas de insectos en la agricultura, y se ha explotado para regular la población de insectos herbívoros (Tibebu, 2018). Los taninos condensados son directamente tóxicos para las plagas de insectos (antibiosis) o disuaden a los insectos de alimentarse (antixenosis) (War et al., 2019). Las defensas constitutivas son cosas que reducen el crecimiento, reducen la producción fotosintética y reducen la aptitud de la planta (Sánchez-Sánchez y Morquecho-Contreras, 2017). Las defensas inducidas incluyen metabolitos secundarios, así como cambios morfológicos y fisiológicos. Una ventaja de las defensas inducibles, a diferencia de las constitutivas, es que sólo se producen cuando se necesitan y, por lo tanto, son potencialmente menos costosas, especialmente cuando la herbivoría es variable (Karban et al., 1997).

El efecto sinérgico entre diferentes componentes defensivos mejora el sistema defensivo de las plantas contra los invasores de insectos. Por ejemplo, en el tomate, los alcaloides, los fenoles, los inhibidores de proteasa (IP) y las enzimas oxidativas cuando se ingieren por separado dan como resultado un efecto reducido, pero actúan juntos de manera sinérgica, afectando al insecto durante la ingestión, la digestión y el metabolismo (Ferrero et al., 2019). En un tabaco silvestre (*Nicotiana atenuado*), los inhibidores de la tripsina proteinasa y la expresión de nicotina contribuyeron sinérgicamente a la respuesta defensiva contra la variabilidad del gusano cogollero (*Spodoptera exigua*) (War, 2012). Cristales y películas epicuticulares de cera cubren la cutícula de la mayoría de las plantas vasculares (Pegadaraju et al., 2005).

En el maíz, la toxicidad de los benzoxazinones y la disuasión hacia el barrenador europeo del maíz, *Ostrinia nubilalis* y los cultivares de trigo con alto contenido de fenol son menos preferidos que el pulgón de los cereales (*Rhopalosiphum padi)* (War et al., 2019). En mostaza negra, la densidad de tricomas y los niveles de glucosinolatos se elevaron mariposa blanca afectada (*Pieris rapae*) (Tibebu, 2018). En tomate, los ataques de áfidos conducen a una mayor atracción de parasitoides de áfidos, mediada por la producción mejorada de compuestos orgánicos volátiles (COV) que se sabe inducen el vuelo de *Aphidius ervi* (Coppola et al., 2017). Cuando se sembró el oloroso *Melinis minutiflora* (hierba de melaza) en campos de maíz, el daño por herbivoría asociado al maíz disminuyó. El pasto emite constitutivamente un compuesto similar al liberado por el maíz en respuesta al daño de las orugas para atraer parasitoides. Las variedades de alfalfa (*Medicago sativa*) que tienen un compuesto fenólico llamado medicarpina, tienen resistencia al nematodo lesionador de raíces (*Pratylenchus penetrans*) (Baldridge et al., 1998). El aumento del número de oxalatos de calcio mejoró la resistencia de los insectos en los cultivares *Prunus avium* contra la plaga *Caliroa cerasi* (Peschiutta et al., 2020). Las orquídeas que se encontraron reclutaron las estrategias defensivas más fuertes, que consisten en una epidermis gruesa, una mayor proporción de cristales de oxalato de calcio en forma de aguja y un mayor contenido de alcaloides y quinonas (Li et al., 2022), lo que indica que las plantas pueden usar uno o diferentes mecanismos de defensa química. Escobar-Bravo et al. (2016) revisaron que los genotipos de tomate silvestre desarrollaron barreras físicas y químicas como mecanismos de resistencia contra la araña roja (*Tetranychus urticae*), incluida la síntesis de acil-azúcares, metilcetonas y terpenoides acumulados en los tricomas, mientras que en los tomates de cultivo estos mecanismos se reducen o se pierden por completo. El aumento del nivel de fenoles totales afectó al áfido verde del durazno, *Myzus persicae*, en pimiento (Mardani-Talaee et al., 2016), *Bemisia tabaci* en berenjena (Hasanuzzaman et al., 2018) y *Nilaparvata lugens* en arroz (Jayasimha et al., 2015).

Las plantas producen una amplia variedad de (aleloquímicos que las protegen de los herbívoros. Estos compuestos pueden reducir el crecimiento, inhibir la reproducción, alterar la fisiología, retrasar la maduración o inducir diversas anomalías físicas o de comportamiento en los herbívoros. Al seleccionar deliberadamente plantas con altos niveles de aleloquímicos, o al cruzar esas plantas con otras menos resistentes, a menudo es posible desarrollar nuevos cultivares que resistan el daño de las plagas y aun así retengan las características deseables del cultivo (War et al., 2019; Smith, 2005). Una propiedad física o química de una planta puede hacerla tan desagradable que está protegida en gran medida del ataque de los herbívoros se llama antibiosis. Este tipo de resistencia a menudo se conoce como no preferencia. Puede involucrar la presencia de repelentes de alimentación (o la ausencia de atrayentes de alimentación), o puede involucrar rasgos físicos como pelos, cerdas o una epidermis gruesa y resistente que obstaculizan a la plaga con un sustrato de alimentación deseable. La alfalfa, por ejemplo, ha sido criada con hojas peludas para evitar que el pulgón manchado de la alfalfa se alimente (War et al., 2019; Smith, 2005). Algunos genotipos de plantas simplemente pueden "tolerar" insectos dañinos mejor que otros. Los cultivares tolerantes pueden estar expuestos a las mismas poblaciones de plagas que los susceptibles, pero no sufren tanto daño (Teetes, 1996).

Generalmente, en plantas infestadas, los insectos herbívoros inducen varias estrategias de defensa. Entre ellos, la defensa química es muy poderosa debido a la enorme cantidad de compuestos diferentes y su alta diversificación estructural, lo que implica un número muy alto de objetivos diferentes en los herbívoros. Las plantas pueden asignar eficientemente la energía y los metabolitos de las formas fijas existentes a los compuestos defensivos necesarios (Mithöfer y Boland, 2012). Los insectos herbívoros pueden superar los efectos negativos de los compuestos defensivos de las plantas empleando diversas estrategias, como la desintoxicación, el secuestro o la secreción (Wielkopolan et al., 2016).

Méndez-Gallegos et al. (2010) observado altas tasas de mortalidad y no generaron descendencia de *D. coccus* en algunas cultivares y sugirió hay mecanismo de resistencia en los cultivares. Este comportamiento puede estar relacionado con aspectos morfológicos (grosor de la cutícula, presencia de oxalatos de calcio) (da Silva et al., 2010) y químicos (presencia de compuestos secundarios) (Akroud et al., 2021). De aquí se puede deducir que existen algunos cultivares resistentes de *O. ficus-indica*, los cuales pueden tener diferentes mecanismos de resistencia. Los estudios realizados hasta ahora dieron una pista de que los *Opuntias* pueden utilizar mecanismos de resistencia tanto bioquímicos como físicos. Akroud et al. (2021) discutieron que la mortalidad podría deberse a las anormalidades que podría ocasionar la ingestión de compuestos, los cuales son insuficientes o antinutritivos para el desarrollo del insecto. El mecanismo de resistencia podría ser la presencia de metabolitos secundarios (War et al., 2020) o barreras físicas como los oxalatos de calcio (Nakata, 2015). López-Palacios y Peña-Valdivia (2020) observaron una mayor abundancia total de ácidos hidroxicinámicos en cladodios de especies silvestres y los autores sugirieron que durante el proceso de selección impuesto se reduce la producción de algunos de estos compuestos debido a que se utilizan variantes domesticadas como hospedantes para el cultivo de cochinilla (*D. coccus*), de donde se obtiene el colorante carmín. Tovar-Puente y Pando-Moreno (2010) afirmaron que la alta concentración de cristales de oxalato de calcio dificulta que las ninfas inserten sus estiletes y se establezcan en los cladodios. Los oxalatos están más concentrados en los cladodios maduros (Contreras-Padilla et al., 2011).

Los metabolitos secundarios se pueden utilizar como biomarcadores para identificar especies (Matos et al., 2021). Las similitudes dentro de la familia, géneros, especies (Isah, 2019). Kaundun y Lebreton (2010) sugirieron para ser utilizado como clasificación e identificación del género *Pinu*s (Pinaceae). Diferencia en contenido de metabolitos secundarios entre especies y variedades *O. ficus-indica* (López-Palacios y Peña-Valdivia,

2020) reportado. La disponibilidad y la concentración depende de la edad genética, ambiental y de crecimiento (Isah, 2019). Las variedades silvestres morado, tempranillo, blanco y cristalino presentaron el mayor contenido de ácido fenólico total (Lemos et al., 2017). Ventura-Aguilar et al. (2017) observaron diferencias en compuestos de polifenoles entre cultivares y el nivel más alto fue en los cultivares silvestres.

Ventura-Aguilar et al. (2017) revisaron que hay variación en grosor de cutícula y epidermis, y también la epidermis contiene cristales de calcio. Los cristales de oxalato de calcio pueden actuar como una barrera contra los insectos, lo que dificulta que los insectos mastiquen esta barrera protectora con muy eficaz (Konyar et al., 2014). De silva et al. (2010) explicaron que la epidermis fue la principal barrera anatómica, proporcionando mayor resistencia e integridad del cladodio y varió entre cultivares. Hudgins et al. (2003) concluyeron que, en los tallos de las coníferas, los patrones y la frecuencia de los cristales funcionan como una defensa constitutiva y, en combinación con las hileras de fibras, proporcionan una barrera eficaz contra los pequeños insectos perforadores de corteza.

Akroud et al. (2021) discutieron que la resistencia de *Opuntia* a *D. opuntiae* podría deberse a las anormalidades que podría ocasionar la ingestión de compuestos, los cuales son insuficientes o antinutritivos para el desarrollo del insecto. Todas las ninfas producidas en genotipos resistentes murieron en el primer estadio, lo que indica que estos genotipos poseen antibiosis como mecanismo de resistencia. Guevara-Figueroa et al. (2010) también agregaron que algunos terpenos afectan el crecimiento y desarrollo de los insectos al interferir con el proceso de muda de la hormona juvenil del insecto. Las saponinas provocan defectos en la muda de insectos o causan toxicidad celular en plagas de insectos (Singh y Kaur, 2018).

El aumento del número de oxalatos de calcio mejoró la resistencia a los insectos en los cultivares *Prunus avium* (Peschiutta et al., 2020). Las orquídeas encontradas reclutaron las estrategias defensivas más fuertes, que consisten en una epidermis gruesa, una mayor proporción de cristales de oxalato de calcio en forma de aguja y un mayor contenido de alcaloides y quinonas (Li et al., 2022), lo que indica que las plantas pueden utilizar uno o diferentes métodos físico químicos. Mecanismos de defensa. Escobar-Bravo et al. (2016) revisaron que los genotipos de tomate silvestre desarrollaron barreras físicas y químicas como mecanismos de resistencia contra la araña roja (TSSM), incluida la síntesis de acil-azúcares, metilcetonas y terpenoides, acumulados en los tricomas.

Se reportan algunos metabolitos secundarios de *O. ficus-indica* con posible defensa contra insectos relacionados con sus parientes. Avila-Niva et al., (2014) identificaron 24 compuestos fenólicos, incluidos compuestos flavonoides de quercetina, kaempferol e isorhamnetina en cladodios de *O. ficus-indica*. *Opuntia* era rica en compuestos antioxidantes naturales como carbohidratos, flavonoides, aminoácidos minerales (arginina), ácidos fenólicos (cafeico y gálico), polifenoles, quercetina, taninos, tocoferol y aminoácidos azufrados (cisteína, metionina y taurina) (Saha et al., 2017). López-Palacios y Peña-Valdivia (2020) observaron una mayor abundancia total de ácidos hidroxicinámicos en cladodios de especies silvestres. Los compuestos relacionados con la defensa de los insectos registrados incluyen hidroxicinámicos, cianidina-Glu, ácido cinámico, procyanidin y malvidina (Rocchetti et al., 2018; Figueroa-Pérez et al., 2018).

Los biomarcadores que posiblemente son responsables de conferir resistencia a las especies de palma forrajera en la cochinilla (*D. opuntiae*) quercetiny rhamnetin (Matos et al., 2021). Ácido 4-hidroxibenzoico (4-HBA), quercetina (QUE) y ácido salicílico (SA). Se detectaron hidroxicinámico, hidroxibenzoico, apigenina, isorhamnetina, quercetina, rutina y ácido oleico en *O. ficus-indica* (de Oliveira et al., 2021). Por lo tanto, aunque no hay un estudio sobre los metabolitos relacionados con la resistencia de *D. coccus*, los estudios sobre las especies relacionadas con la resistencia de *D. opuntiae* y la resistencia de *D. coccus* (en *O. robusta*) pueden dar pistas para apuntar a algunos metabolitos característicos de cultivares resistentes de *O. ficus-índica*. Estos incluyen, entre otros, ácido 4-hidroxibenzoico, ácido clorogénico, quercetina, ácido salicílico, hidroxicinámico, apigenina, isorhamnetina, ramnosil dihexósido, rutina, ácido oleico y otros. Idiomas, fenólicos, flavonoides, fitoestereoles, saponinas; y enzimas (Figueroa-Pérez et al., 2018); Alcaloides (Gibson y Horak, 1978). La disponibilidad y la concentración dependen de la edad genética, ambiental y de crecimiento (Isah, 2019). Existe una cantidad abrumadora de metabolitos secundarios como terpenoides, alcaloides, fenólicos, glucosinolatos y glucósidos cianogénicos, que tienen un rol crucial en la defensa de las plantas contra herbívoros. El conocimiento sobre la presencia, eficiencia y modo de acción de los compuestos especializados efectivos contra los herbívoros es un requisito previo (Mithöfer y Boland, 2012).

1.6 Micropropagación y calidad como forraje de cultivares *Opuntia ficus indica* resistente a *Dactylopius coccus*

La propagación *in vitro* tiene diferentes ventajas (Lema-Ruminska y Kulus, 2014) como disponibilidad, viabilidad, y para satisfacer dicha demanda (Orozco et al., 2007). Esta planta produce un gran número de nuevas plantas de alta calidad en un tiempo y espacio relativamente cortos (Lema-Ruminska y Kulus, 2014). La propagación *in vitro* es muy eficiente en esta familia, y las plantas generadas de esta manera se pueden utilizar para varios propósitos, reduciendo así la presión sobre las poblaciones silvestres causada por la colecta furtiva (Pérez-Molphe-Balch et al., 2012). Los cultivares resistentes deben ser accesibles para los agricultores y un mecanismo para ello es la multiplicación masiva mediante cultivo *in vitro* (El Finti et al., 2013).

Opuntia ficus-indica se puede micropropagar con éxito mediante areolas y se aclimata fácilmente a las condiciones del campo, por lo que representa la mejor herramienta estratégica para combatir la desertificación en regiones áridas y semiáridas (Khalafalla, 2007). Es posible un sistema exitoso de micropropagación y regeneración de plantas completas para varios genotipos de *Opuntia* utilizados como fuentes vegetales para el consumo humano (Garcia-Saucedo et al. 2005). La micropropagación a través de la brotación de yemas axilares es ampliamente utilizada para asegurar la homogeneidad y estabilidad genética y producir verdaderos clones de plantas (Bouzroud et al., 2022).

La alimentación animal se encuentra entre los usos más importantes del nopal en diferentes países como Tigray (Etiopía) (Brutsch, 1997), Brasil (Batista et al., 2009; de Sá Souza et al., 2022) y México (Valdez y Osorio, 1997). El nopal tiene un buan calidad de carbohidratos para animales (Vieira et al., 2008; Batista et al., 2009El uso de nopal como forraje para el ganado en regiones semiáridas de Brasil también fue elaborado por de Andrade Ferreira et al. (2012). posiblemente originario del Altiplano Central de México (Cervantes-Herrera et al., 2006) es resistente al *D. coccu*s (Berhe et al., 2022). Rössel-Kippin et al. (2021) explicaron que los cultivares ‘Copena’, ‘Pelón Blanco’ y ‘Rojo Pelón’ de *O. ficus-indica* comprenden la mayor superficie de cultivo registrada en el altiplano potosino. Este cultivar se utiliza principalmente para la obtención de frutas, dado que no tiene espinas, los cladodios maduros son valiosos como alimento para el ganado (Cervantes-Herrera et al., 2006).

1.7 Hipótesis

Los cultivares de *Opuntia ficus-indica* (L.) Mill. resistentes a *Dactylopius coccus* Costa tienen propiedades físico-químicas (estructuras histológicas y metabolitos secundarios) responsables de dicha resistencia.

1.8 Objetivos

General:

Determinar mecanismos de resistencia en cultivares de *Opuntia ficus-indica* a *Dactylopius coccus.*

Particulares:

1. Evaluar la infestación de cultivares de *O. ficus-indica* a *D. coccus*.
2. Identificar las estructuras histológicas de cultivares de *O. ficus-indica* que dan resistencia contra *D. coccus*.
3. Identificar los metabolitos secundarios de cultivares de *O. ficus-indica* que dan resistencia contra *D. coccus*.
4. Adaptar protocolos de micropropagación y de análisis de calidad como forraje de los cultivares resistentes.

1.9 Justificación

Se cubren algunos estudios sobre cultivares resistentes de *O. ficus-indica* a *D. coccus*. Por lo tanto, los estudios de evaluación y tamizaje son muy importantes. Además, no estudie sobre los mecanismos de resistentes de los cultivares de *Opuntias* cuales resistentes a *D. coccus*. Según estudios similares sobre *Opuntias* resistentes a *D. opuntia*, estos pueden ser mecanismos físicos o metabolitos secundarios (Molano-Flores, 2001; Nakata, 2015; War et al., 2020), lo que lleva a la necesidad de experimentos exploratorios. Además, se identifican muchos metabolitos secundarios en especies de *Opuntia* (Guevara-Figueroa et al., 2010; Lemos et al., 2017; Astello, 2014; López-Palacios y Peña-Valdivia, 2020), pero se desconoce su relación con la defensa de *D. coccus*. Estudios previos indicaron que el oxalato de calcio, el grosor de la cutícula y el grosor de la epidermis pueden contribuir a la defensa de *D. opuntie* en los cultivares de *Opuntia* (De silva et al., 2010; Konyar et al., 2014; Escobar-Bravo et al., 2016; Ventura-Aguilar et al., 2017; Li et al., 2022), Sin embargo, no hay estudios sobre cultivares resistentes a *D. coccus*.

El nopal se puede cultivar *in vitro*, pero existe diferencia entre variedades, observada en respuesta a los reguladores de crecimiento, como ejemplo, 0.5 mg/L de benciladenina (BA) fue lo mejor para la formación de yemas (García-Saucedo et al., 2005). En otro ejemplo, la proliferación máxima de brotes se obtuvo cuando explantes se cultivaron en medio MS suplementado con 5 mg/L de BA (El Finti et al., 2013). Aliyu y Mustapha (2007) encontraron más brotes de *O. ficus-indica* con 1.25 mg/L de BA y 0.25 mg/L de ácido indolacético (AIA). García-Saucedo et al. (2005) también reportaron que 0.5 µM de BA para un mejor número de brotes. Por otro lado, se han registrado diferencias en el número de brotes, la longitud de los brotes, el número de raíces y la longitud de las raíces entre seis genotipos en Tigray (Mengesha et al., 2016) y tres genotipos en Marruecos (El Finti et al., 2013), por lo que las variedades también pueden responder de manera diferente a las condiciones del medio, lo que indica la importancia de optimizar las condiciones de crecimiento para maximizar el valor de diferentes cultivares (Bougdaoua y El Mtili, 2020). Los diferentes protocolos para diversas especies y clones de cactáceas deben utilizar protocolos de micropropagación específicos (Lema-Rumińska y Kulus, 2014; Soltero y Portillo, 2005). Por lo tanto, es importante protocolizar los experimentos de adaptación para identificar mejor tipo y tasa de hormona de crecimiento para agregar a los medios de crecimiento.

Sin embargo, es necesario determinar la calidad nutricional importante, como la calidad de la proteína y el contenido de minerales de algunos genotipos de nopal (Ben Salem y Abidi, 2009). Junto con el desarrollo de resistencia a las plagas, el contenido de nutrientes y la palatabilidad también son aspectos importantes a incluir durante el mejoramiento de nopales (Mondragón-Jacobo y Peréz-Gonzalez, 2002; Ben Salem y Abidi, 2009), ya que la composición nutricional puede ser afectados por cultivares o genotipos (Batista et al., 2009). El cultivar 'Rojo Pelón' que es resistente a *D. coccus* necesita ser probada su calidad nutricional forrajera *in vivo*.

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CAPÍTULO 2. RESISTENCIA DE *Opuntia ficus-indica* PARA *Dactylopius coccus* COSTA (HEMIPTERA: DACTYLOPIIDAE)

2.1 Resistencia de *Opuntia ficus-indica* cv 'Rojo Pelón' a *Dactylopius coccus* (HEMIPTERA: DACTYLOPIIDAE) bajo condiciones de invernadero.

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Resistance of *Opuntia ficus-indica* cv 'Rojo Pelon' to *Dactylopius coccus* (Hemiptera: Dactylopiidae) under greenhouse condition

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Abstract. *Opuntia ficus-indica* (L.) Mill.), is called "beles" in the Tigray region of Ethiopia, where it is used for multiple purposes, such as food, fodder, and fences; however, in recent years the introduction of the cochineal (*Dactylopius coccus* Costa) to this region has caused it to become a plague that has affected thousands of hectares, causing an economic and social problem. Six cultivars: three from *Opuntia ficus-indica* ('Atlixco', 'Chicomostoc', and 'Rojo Pelón') and three others *O. cochenillifera* ('Nopalea' and 'Bioplástico') and *O. robusta* ('Robusta') were tested for resistance to *D. coccus* in completely randomized design (CRD) experiment with six replications under greenhouse conditions, for two seasons (cycles). Matured cladodes were infested by attaching a paper bag with ten ovipositing female *D. coccus*. The number of insects at different stages and yields were recorded, log transformed (insect count data), and subjected to statistical analysis. The number of nymphs (stages I and II) was significantly different in both cycles (P=0.0000). The insects died at the nymph I stage at 'Rojo Pelón' and 'Robusta', in contrast, they completed their life cycle at 'Atlixco', 'Chicomostoc', and 'Nopalea' cultivars. Insects at 'Bioplástico' cultivar remained in the nymph I stage the whole experimental period; the molting was hampered. Although some crawlers started settling (nymph I) at 'Rojo Pelón', they couldn't survive and developed, and this confirms that this *O. ficus-indica* cultivar is resistant to *D. coccus*.

Keywords: *Beles, cactus pear, cochineal, resistant cultivar*

Introduction

Opuntia ficus-indica (L.) Miller (cactus pear) is among the Cactaceae family, which has about 127 genera of which 1750 are identified species of the order Caryophyllales (Christenhusz and Byng, 2016). During the XVI century, *O. ficus-indica* was an important commodity in Mexico (Reyes-Agüero *et al.*, 2005), and because of the different benefits this species had, like morphological peculiarities, anti-scurvy properties, sweet edible fruits, and ability to host the cochineal insect, from which an important commodity called cochineal dye was gained, the Spanish took it to their homeland. Later it was expanded to other regions of the world, including the Mediterranean area (Barbera *et al.*, 1992; Griffith, 2004). It was also introduced to Tigray, around themiddle XVIII century (Neumann 1997; cited in Gebretsadik *et al.*, 2013). The cactus pear, locally called beles in the Tigirigna Language of Ethiopia, is a crop that is used in some parts of Tigray, as a source of human food for 4 or 5 months and for livestock feed, and its isconsidered an emergency crop. Although it can grow in different areas, it is obvious that beles can grow better in specific areas (Lemma *et al.*, 2010). It is appreciated for its various benefits, which include high biomass yield, growth in sandy soils, staying greenthroughout the year, and resistance to drought; this suggests a high potential for use for different other purposes (Brutsch, 1997). In Mexico this plant is known as "nopal", but in other countries is "tuna", "cactus pear", "prickly pear", "green jewel" "green gold", "fruit for the poor", "treasure under its thorns", "world plant dromedary", "future plant", "sacred plant", and "monstrous tree" (Arias-Jimenez, 2013).

Cactus pear in North Africa, Brazil, and the Middle East is at risk from the wild cochineal (*Dactylopius opuntiae* (Cockerell)) (Bouharroud *et al.,* 2016; Mazzeo *et al.*, 2019; Torres and Giorgi, 2018). In Ethiopia, the fine cochineal insect (*D. coccus* Costa) has caused damage in several areas of the Tigray region (Belay, 2015; Berhe *et al.*, 2020). Due to the aforementioned reasons, it is necessary to carry out research to combat this problem with some options including the utilization of resistant varieties.

Dactylopius species or biotypes prefer specific species of *Opuntia* as a host and parasitize only cacti (Volchansky *et al.*, 1999; Hoffmann *et al.*, 2002: Campana *et al.*, 2015; Portillo and Vigueras, 2006). The breeding of *D. coccus* is carried out in a small number of nopal species, it has been reported that only 10% of the total number of opuntias are used; among these species, *O. ficus-indica* is with its different cultivars, which is preferred by producers due to its ease of handling (few spines), larger cladodes, in addition to being used for other purposes such as the food and forage (Campana *et al.*, 2015; Van Dam *et al.*, 2015; Vigueras and Portillo, 2014). *D. coccus* is a species cultivated worldwide (Portillo and Vigueras, 2006; Van Dam and May, 2012), which produces the pigment called carminic acid and which is processed in different presentations such as lacquers and carmine (Piña, 1979; Vigueras and Portillo, 2014). This dye is one of the most demanded and has replaced artificial dyes (red); it is used as an additive for food, medicines, cosmetics, and textiles (Arroyo-Figueroa *et al.*, 2016; Torres-Ponce *et al.*, 2015; Arroyo-Figueroa *et al.*, 2009); and it is preferred for its high stability to light and temperature, in addition to being harmless to human health (Vigueras and Portillo, 2014).

But it became invasive and a pest of the host *O. ficus-indica* in Tigray, because of different reasons; like the absence of natural enemies, dense plant populations, and in-proper management (Belay, 2015). Having long and short-term management plans are important to control insect and integrated pest management (IPM) gives more emphasis on environmental pollution and the well-being of human being. Resistant varieties are an important component of the IPM to manage the infestation in Tigray (Berhe *et al.*, 2020).

Although the development or evaluation of resistant cultivars of *O. ficus-indica* to *D. coccus* is rarely reported, the utilization of pest resistant crop varieties is important for the economy, environment, and ecology. The economic advantages are reducing crop loss due to insects and minimizing the cost of insecticides. It is also easily available to small-scale growers, especially in developing countries. Mostly, the seed of insect-resistant varieties are a little more expensive than the cost of the susceptible varieties or sometimes can be equal (Teetes, 1996; Huang *et al.*, 2013). More importantly, farmers don't require any special skills or techniques of application (Sharma and Ortiz, 2002). It also helps to reduce the use of chemicals and indirectly related risk of environmental pollution and human health (Munhame *et al.*, 2021; Reinert *et al.*, 2003; Vigueras y Portillo, 2014). The genus *Opuntia* has higher diversity and distribution in the Americas than the other Cactaceae (Reyes-Agüero and Valiente-Banuet, 2006). There is a high genetic similarity between *O. ficus-indica* and *O. megacantha* and it indicates *O. ficus-indica* that could be a domesticated type of *O. megacantha* (Kiesling and Metzing, 2017). *O. ficus-indica*, *O. albicarpa*, and *O. megacantha* possibly share an ancestry (Valadez-Moctezuma *et al.*, 2015). Some of the wild species are resistant to *D. coccus* and the genetic similarity of *O. ficus-indica* with the different species implies that; there could be genetic sharing hence some *O. ficus-indica* cultivars may have resistance capability. Plants have evolved a whole arsenal of defense strategies against pests, including morphological structures that form the first line of protection to capacity in synthesizing an enormous array of chemical compounds (War *et al*., 2020).

Tovar *et al.* (2005) reported that *D. coccus* yield obtained from var. Villanueva is higher than varieties tested in Mexico. Méndez-Gallegos *et al.* (2010) explained that 'Blanco Moscatel' and 'Colorado Moscatel' cultivars might have defense means for *D. coccus*, since the colony development on those hosts showed a high mortality rate and failed to reproduce. This behaviour may be associated with the presence of phytochemicals (such as terpenoids, flavonoids, tannins, and polyphenols) (Akroud *et al*., 2021; Guevara-Figueroa *et al*., 2010; Matos *et al*., 2021) and morphological-histological characteristics (epidermis and cuticle thickness and density of calcium oxalates) (da Silva *et al*., 2010). Host resistance should be considered in cochineal control (Vigueras and Portillo, 2014). Thus, it can be possible to find resistant cultivars which may have similar properties to resistant wild species, and exploration and verification are required. Identification of resistant varieties and a deeper understanding of the levels and mechanisms of resistance is vital for integrated pest management strategies (War *et al*., 2020). It is also imperative paramount to study the genetics of *O. ficus-indica* such as identifying the morphological resistance characteristics of genotypes and finding the genes that control resistance to cochineal (Felker and Inglese, 2003). Resistant varieties can be used either as the main method or as a supplement to other strategies of insect control, based on their resistance level (Jayaraj and Uthamassamy, 1990). Therefore, the research was conducted to assess the cultivar 'Rojo Pelon' of O. *ficus-indica* resistance to *D. coccus* for further utilization and breeding purposes.

Materials and Methods

The experiment comprised six *Opuntia* cultivars collected from different production farms and locations in Mexico: three *O. ficus-indica* ('Rojo Pelón', 'Atlixco', and 'Chicomostoc'), two *O. cochenillifera* ('Nopalea' and 'Bioplástico') and one *O. robusta* ('Robusta') (Table 1). For the infestation of the cladodes, females ovipositing was used (where nymphs were observed), this material was obtained from cochineal breeding in greenhouse. It was carried out at the University of Guadalajara, Center of Biological and Agricultural Sciences (CUCBA), Zapopan, Jalisco, Mexico, under greenhouse conditions and with a completely randomized design (CRD) replicated six times (cladodes as an experimental unit) in two seasons/cycles (Cycle 1: October-January 2020/2021 and Cycle 2: January-April 2021), since these two seasons have different climatic conditions. At Cycle 1 temperatures were 10 to 38.05°C and relative humidity between 36.95 to 56.77%; meanwhile, for Cycle 2 the records ranged from 12.3 to 45.82°C and relative humidity 10 to 66.95%.

The climatic conditions from where the hosts were collected: Ojuelos, Jalisco has an average annual temperature of 13.9°C, and 594 mm rainfall (IIEG, 2021). Guadalajara has an average temperature of around 20.9°C and 900 mm rainfall; Villanueva, Zacatecas has an annual temperature of 17°C, annual mean rainfall of 510 mm (INEGI, 2022).

Table 1. Identity and cladode characteristics (cultivar name, species name, national ID, source, and presence of spines) of the six *Opuntia* cultivars evaluated.

Cultivar name	Species name	National ID	Source	Spininess
Rojo Pelón	*O. ficus-indica*	NOP-022-221104	OjJ	Spineless
Atlixco	*O. ficus-indica*	NOP- 018-221104	VNZ	Spineless
Chicomostoc	*O. ficus-indica*	NA	VNZ	Spineless
Bioplástico	*O. cochenillifera*	NA	VNZ	Very few
Nopalea	*O. cochenillifera*	NA	GDLJ	Spineless
Robusta	*O. robusta*	NOP-060-090617	OjJ	Spiny

Note: OjJ-Ojuelos, Jalisco, VNZ-Villanueva, Zacatecas, GDLJ-Guadalajara, Jalisco. Source: CNVV, 2020.

All selected matured cladodes were one-year-old, vigorous, and free of plagues and diseases. Cladodes were thoroughly washed with pure water for cleaning from any wild cochineal (*D. opuntiae*), their length, width, and thickness were measured with a vernier caliper and a weighing balance was used to measure their weight. Then, the cladodes were labelled and marked for repeat counts, hanged in reverse position, in such a way that they were pierced at their base with wire and placed on a metal barrel. For the infestation of the cladodes, females close to oviposition were used (when nymphs were observed), from this material was obtained from the cochineal breeding in the greenhouse. The adult females were collected (Figure 1a) from the source host cladodes with a fine brush and kept in small paper bags which were fixed with spines (Figure 1b) containing ten adult females each. Infested cladodes were covered with nylon clothing bags (60 cm x 30 cm size) to protect them from uncontrolled infestation and pests (Figure 1c). The paper bags remained attached to the cladodes for 7 days and then removed (Gusqui Mata, 2013). After the females started oviposition (matured), they were harvested with a fine brush to take the yield data.

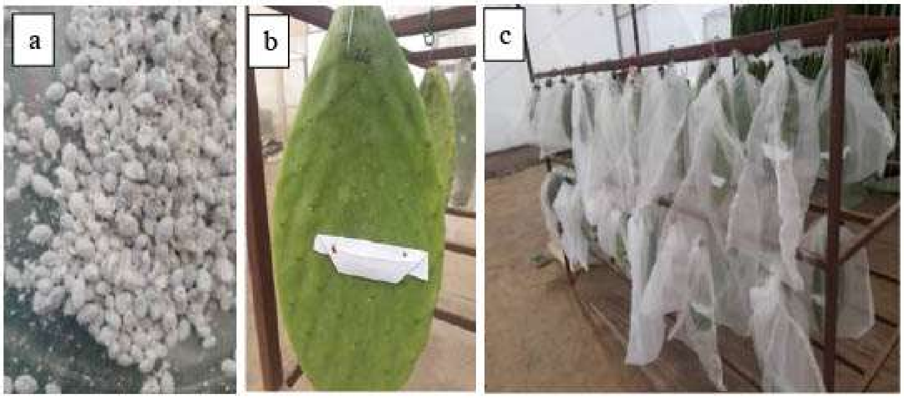

Figure 1. Infestation of cladodes. a) female ovipositing cochineal ready for infestation, b) fixing paper bags that contain female ovipositing cochineal to the cladodes, c) infested cladodes covered with nylon clothing bags.

Data were collected on insect counts including the number of crawlers (mobile nymph I), nymph I established, nymphs II, cocoons, and female adults; total fresh and dry weight per cladode (g); the fresh and dry weight of individual adult female (mg) from each experimental unit (cladode). To take the individual weight, ten samples were randomly weighted, and the average was recorded. Dry weights were measured after the insects were dried in an oven at 60°C for 3 h. Data were checked for homogeneity, transformed with log (count data) and square root (number of cocoons), and an analysis of variance (ANOVA) was performed using the statistical package R.4.1.0. Tukey test at (0.05) was applied to compare means among treatments if significant differences were detected.

Results and Discussion

Cladode characteristics of the cultivars used

Cultivars used in cycle 1 and 2 had significant differences (P = 0.0000 both) in cladode weight (g), width (cm), and thickness (cm), and (P = 0.0173 and P = 0.0011) in length (cm) (Table 2). In line with this finding, Peña-Valdivia *et al.* (2008) reported differences in morphological characteristics of *opuntias*, and presence of spines on the surface of cladodes, and cladodes' width and length. Adli *et al.* (2019) also observed significant differences in cladode length, width, and thickness among four *O. ficus-indica* accessions. Cladode shapes may be considered important parameters for taxonomic classification even to differentiate forage cacti (Lucena *et al.*, 2019). Morphological characteristics of cladodes (height, width, weight, and thickness) can have effects on the yield of cochineal (Arroyo-Figueroa *et al.*, 2020).

Table 3. Means of cladode weight (CWt) in g, length (CL) in cm, width (CW) in cm, and thickness (CT) in cm in cycle 1 (C1) and cycle 2 (C2).

CV	CwtC1	CwtC2	CLC1	CLC2	CWC1	CWC2	CTC1	CTC2
RP	1128.33^{d}	932.5000^{c}	30.67^{b}	27.8333^{bc}	17.33^{b}	16.3333^{bc}	3.50^{d}	3.3333^{c}
A	467.50^{a}	705.0000^{bc}	30.67^{b}	31.3333^{c}	12.83^{a}	14.1666^{ab}	2.04^{ab}	2.2500^{ab}
C	741.67^{b}	591.6667^{bc}	31.33^{b}	29.5000^{bc}	18.50^{b}	17.8333^{c}	2.04^{ab}	1.9583^{ab}
B	476.67^{ac}	375.0000^{ab}	28.67^{ab}	26.3333^{bc}	12.50^{a}	11.0000^{a}	2.42^{bc}	2.2916^{ab}
N	247.50^{a}	238.3333^{a}	31.17^{b}	24.6666^{ab}	10.50^{a}	11.1666^{a}	1.46^{a}	1.8333^{a}
R	836.67^{c}	594.1667^{bc}	22.33^{b}	23.0000^{a}	19.33^{b}	17.1666^{bc}	2.96^{cd}	2.5416^{b}

Means sharing the same letter are not significantly different.

CV-cultivar,RP='Rojo Pelón', A='Atlixco', C='Chicomostoc', B='Bioplástico', N='Nopalea', and R='Robusta'.

D. coccus survival and development

Statistically, a significant difference was observed among the cultivars in the number of crawlers/mobile nymph I (NC) in cycle I (P = 0.0398), but not in cycle 2 (P = 0.1520), where the number was higher in 'Chicomostoc' but statistically similar with 'Atlixco' and 'Nopalea'. The number of nymphs I (NI) indicated a significant variation at both cycles (P=0.0000). Additionally, for both cycles, there was a statistically significant difference in the number of nymph II (NII) (P=0.0000). At cycle 2, a lower number of nymphs I was observed on 'Rojo Pelón', and a higher number was observed on 'Atlixco' (but statistically like 'Chicomostoc'). The number of nymphs I was significantly similar within the resistants ('Robusta', 'Rojo Pelón', and 'Bioplástico'). The number of nymphs II was statistically similar among the susceptible cultivars, but there was no nymph II observed in the resistant cultivars (Figure 2).

The similarity of *Opuntia* cultivars in the number of crawlers is an indication of the insects' non-preference for oviposition. The difference in development among different cultivars is similar to previous reports. Passos da Silva *et al.* (2007) found a difference in resistance among *Opuntia* clones to *D. opuntiae*. Sbaghi *et al*. (2019) also reported that seven resistant ecotypes out of 241 tested proved to be resistant to *D. opuntiae* pest in Morocco.

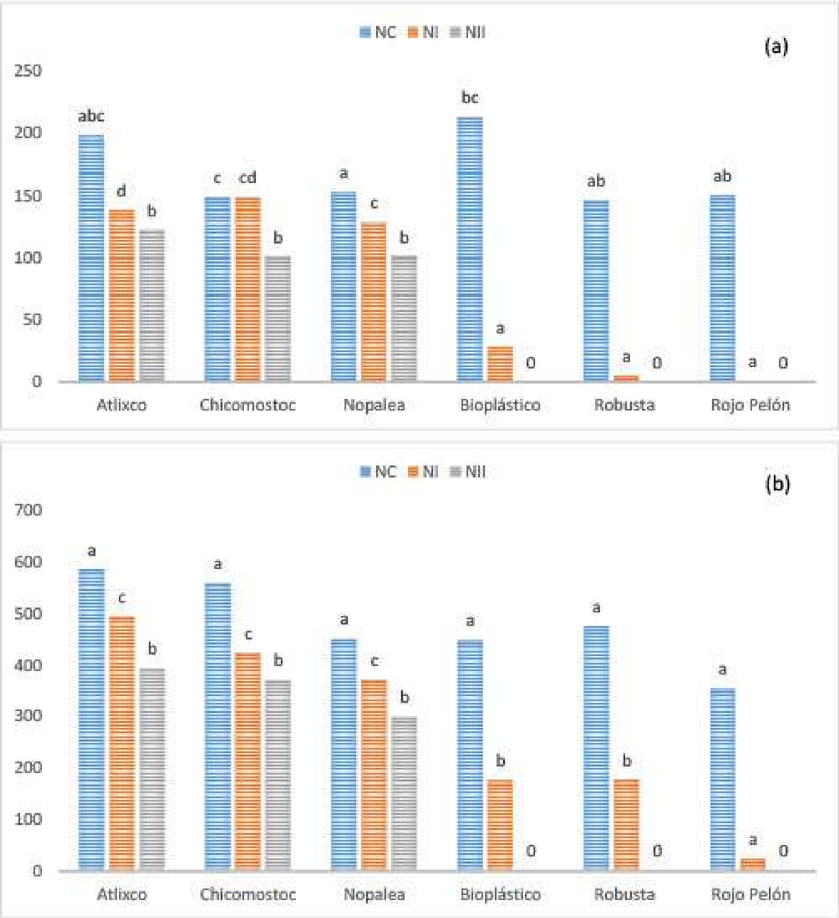

Figure 2. Development of *Dactylopius coccus* on six *Opuntia* cultivars. The number of crawlers (NC), nymphs I (NI), and nymphs II (NII) at cycle 1 (a) and cycle 2 (b). Means sharing the same letter are not significantly different.

It was noted that the insects died at the nymphs I stage, after 21 days of infestation on two resistant cultivars ('Robusta' and 'Rojo Pelón') (Figure 2). Growing nymphs II with white wax on 'Atlixco' (Figure 3a) and insect-free on 'Rojo Pelón' (Figure 3b).

(a) Atlixco (b) Rojo Pelón

Figure 3. Status of insects in *Opuntia* cultivars after 21 days after infestation. a) susceptible cultivar ('Atlixco') with *Dactylopius coccus* showing covered with white wax, b) resistant cultivar ('Rojo Pelón') without *D. coccus*.

Opuntia cultivars also showed statistically significant differences (P=0.0000) in the percentage of crawler mortality and nymph I mortality at both cycles at $p \leq 0.05$. Crawlers' mortality was higher in the resistant cultivars (86 % to 96%) than in susceptible cultivars (28% to 35% in cycle 1). In cycle 2, crawlers' mortality was higher at 'Rojo Pelón' (100%) followed by 'Robusta' (94%), and 'Bioplástico' (63%). Similarly, nymph I mortality was higher (100%) in resistant cultivars, 'Rojo Pelón', 'Bioplástico', and 'Robusta' (Figure 4). In agreement with this finding, nymph mortality of *D. opuntia* was high, ranging from 40 to 60% at different cultivars, during the first 24 days of development (Méndez-Gallegos *et al*., 2010). Akroud *et al*. (2021) found that mortality of *D. opuntiae*, started on the 7th day after infestation and maximum mortality on the 28th day. The authors also discussed that the mortality might be due to the abnormalities that could be caused by the ingestionof compounds (secondary metabolites like alkaloids, carotenoids, diterpenes, amines, etc.), which are either insufficient or anti-nutrition for the insect's development. At the two resistant cultivars, all nymphs died at first instar which may be due to the cultivars possessing antibiosis mechanisms of resistance. This finding can suggest that 'Rojo Pelón' is a resistant cultivar to *D. coccus*. Similarly, Sbaghi *et al*. (2019) and Passos da Silva *et al*. (2007) concluded that five cultivars proved to be immune to the *D. opuntiae*, since there has been no insect development.

Adult and cocoon populations showed a significant difference across cultivars (P=0.0000) at both testing cycles, but there was no statistical difference among susceptible cultivars (Table 3). Cultivars had no significant difference in total insect fresh weight at both cycles (P=0.5540 and 0.0744), total dry weight at cycle 1 (P=0.7860), individual fresh weight at cycle 2 (P=0.3680), and individual dry weight at cycle 2 (P=0.3320). However, there were statistically significant differences in individual insect fresh and dry weight (P=0.0003 and P=0.0007) at cycle 1 and total dry weight at cycle 2 (P=0.0475) (Figure 5). 'Atlixco' followed by 'Chicomostoc' cultivars were superior to 'Nopalea' in insect performance, measured by the above parameters (Table 3 and Figure 5). These results support the conclusions made by Tovar *et al*. (2005) who noticed significant differences among cultivars. Similarly, Méndez-Gallegos *et al*. (2010) reported differences in the carminic acid percentage of *D. coccus* among different cultivars. Arroyo-Figueroa *et al*. (2020) also reported the effect of morphological characteristics in cladodes of different ages, such as length, width, and weight affected *D. coccus* yield; which can be also different intra and inter-species (Peña-Valdivia *et al*., 2008; Adli *et al*., 2019).

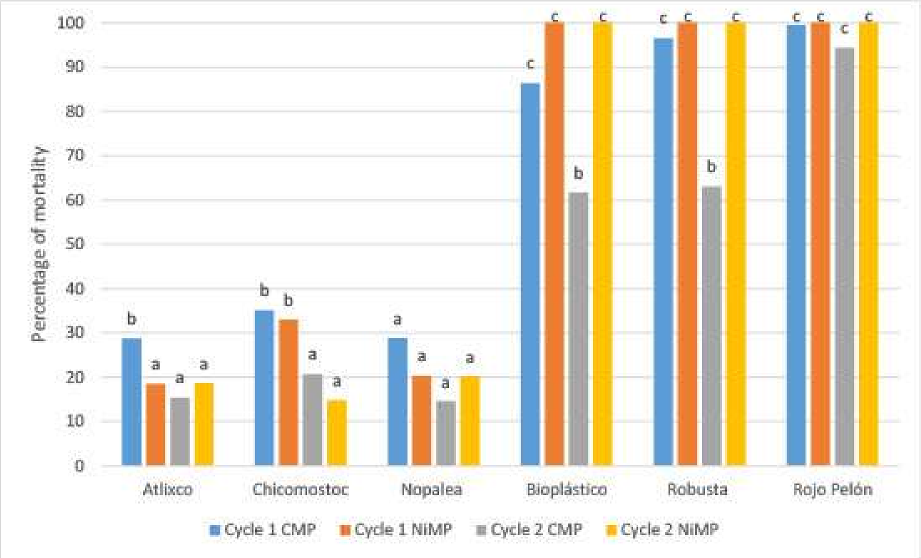

Figure 4. Mortality of nymphs (percentage) on six *Opuntia* cultivars. Mortality of crawlers at cycle 1 (cycle 1 CMP) and cycle 2 (Cycle 2 CMP), mortality of nymph I at cycle 1 (Cycle 1 NiMP) and cycle 2 (Cycle 2 NiMP) at $p \leq 0.05$. Means sharing the same letter are not significantly different.

Table 3. Means of the number of adults (females) and cocoons of *Dactylopius coccus* on six *Opuntia* cultivars at cycle 1 (C1) and cycle 2 (C2).

Cultivar	**Adults C1**	**Cocoons C1**	**Adults C2**	**Cocoons C2**
'Rojo Pelón'	0^{a}	0^{a}	0^{a}	0^{a}
'Atlixco'	49.17^{b}	14.83^{b}	255.83^{b}	66.33^{b}
'Chicomostoc'	49.00^{b}	18.00^{b}	201.50^{b}	54.17^{b}
'Bioplástico'	0^{a}	0^{a}	0^{a}	0^{a}
'Nopalea'	55.67^{b}	14.50^{b}	148.17^{b}	37.50^{b}
'Robusta'	0^{a}	0^{a}	0^{a}	0^{a}

Note: Means sharing the same letter are not significantly different.

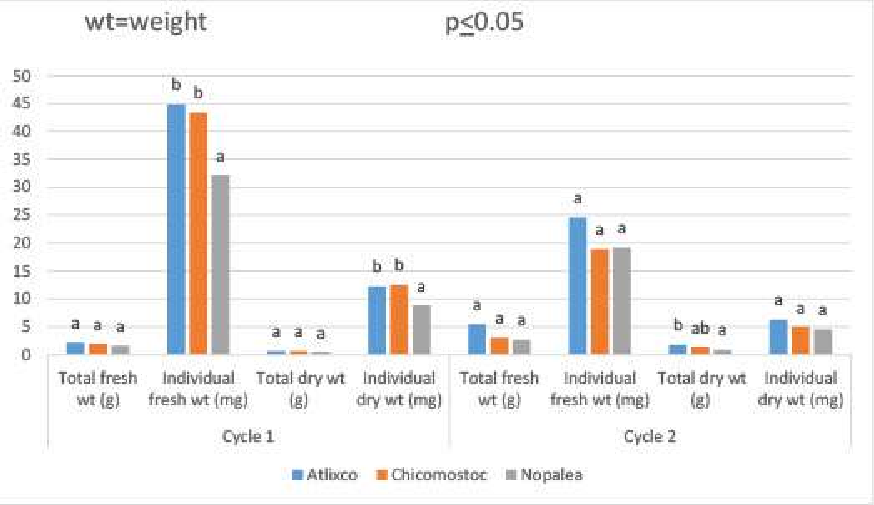

Figure 5. Insect fresh and dry weight total (g), fresh and dry weight individual (mg), at cycles 1 and 2.

The number of days to complete the female life cycle of the insect (harvesting) showed a statistically significant difference among the susceptible cultivars (P=0.0000). *D. coccus* completed its life cycle earlier at 'Atlixco' followed by 'Chicomostoc', and 'Nopalea' (Figure 6). Overall, the days taken to maturity are in the range of the life cycle of the insect. In accordance with this, Arroyo-Figueroa *et al.* (2020) explained that *D. coccus* needs 90 to 120 days to complete its life cycle. Méndez-Gallegos *et al.* (1993) also support that the period to maturity of *D. coccus* is around 90 days.

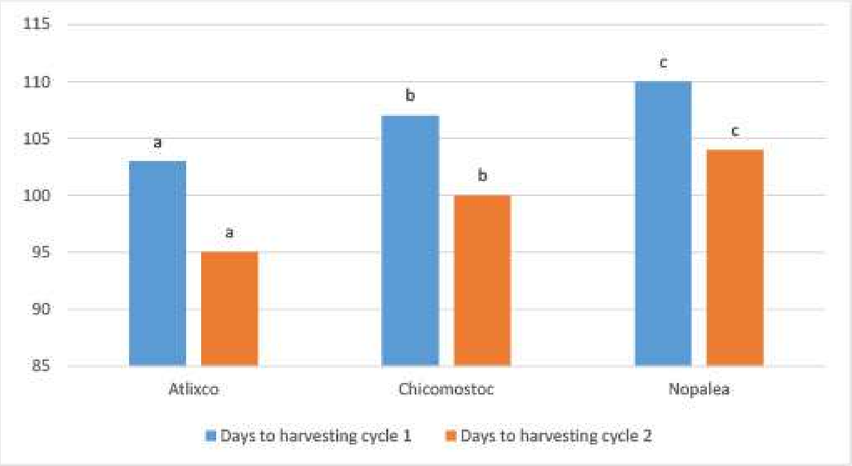

Figure 6. Days to harvesting of *Dactylopius coccus* under three susceptible cultivars ('Atlixco', 'Chicomostoc', and 'Nopalea'). Means sharing the same letter are not significantly different.

Insects completed their life cycle on the three susceptible cultivars ('Atlixco', 'Chicomostoc', and '*Nopalea*'). In 'Bioplástico' the insects remain in nymph I (Figure 7). At cultivars ('Robusta' and 'Rojo Pelón') the insects died during the first instar (nymph I) (Figures 4 and 7). The susceptible hosts are members of *O. ficus-indica* which are the commonly used hosts for cochineal production (Campana *et al*., 2015). The mechanism of resistance could be the presence of secondary metabolites (War *et al*., 2020) or physical barriers like calcium oxalates (Nakata, 2015; Molano-Flores, 2001). López-Palacios and Peña-Valdivia (2020) observed higher levels of hydroxycinnamic acid in the cladodes of wild species. Tovar-Puente and Pando-Moreno (2010) high concentration of calcium oxalate crystals stated that prevents the nymph from inserting the stylet and settling in the cladode. Oxalates are more concentratedon matured cladodes (Contreras-Padilla *et al*., 2011).

Figure 7. Infestation status of all six cultivars at the end of the experiment periods. a='Nopalea', b='Atlixco', c= 'Chicomostoc', d='Robusta', e='Rojo Pelón', and f= 'Bioplástico'. Susceptible cultivars a, b, and c with matured *Dactylopius coccus* and resistant cultivars d and e free of insects and f with nymphs (arrow).

Generally, the findings are supported by previous works. Tovar *et* al. (2005) and Méndez-Gallegos *et al*. (2010) reported differences in *D. coccus* performance among cultivars in Mexico and suggested the possibility of resistance. Sbaghi *et al*. (2019) also found seven ecotypes shown to be resistant to *D. opuntiae* in Morocco. Since there has been no pest establishment, a cultivar could be resistant; hence, our results confirm that 'Rojo Pelón' is a promising resistant cultivar of *O. ficus-indica* to *D. coccus*. The host and the insect have obviously been in continuous adaptations and many wild species are resistant to these insects; so, it is inferred that this domesticated cultivar may share some genetic characteristics with some of the wild species.

The 'Rojo Pelon' cultivar probably originated from Northern Guanajuato and Southern San Luis Potosi (Central highlands of Mexico). Characterized by an elliptical shape, bright red fruit colour, and sweet export quality fruit that matures during the summer season. The tender and spineless cladodes it has been important for vegetable and livestock feed (Cervantes-Herrera *et al.*, 2006).

Remaining the insects in the nymph I stage throughout the experimental period of the complete life cycle on 'Bioplástico' cultivar (Figure 6) implies moulting was hindered, probably by some Phyto-ecdysones (terpenoids) (War *et al.*, 2020). Moulting growth can be continuous, as in hemimetabolous insects (e.g., aphids, cockroaches, grasshoppers, and locusts) or resting pupal stage, when the larval organs are completely changed to adult level (holometabolous insects, e.g., beetles, moths, flies, and ants) (Morgan and Poole, 1977). It is under the control of the brain (the corpus cardiacum) (Veelaert *et al.*, 1998) that stimulates the prothoracic gland to secrete moulting hormone or its precursor (Krishnakumaran and Schneiderman, 1968; Kobayashi and Yamazaki, 1974; Ishizaki and Suzuki, 2002).

Conclusions

The evaluated cultivars belong to three species of *Opuntia*, three *O. ficus-indica* cultivars, one *O. robusta* cultivar, and two *O. cochenillifera* cultivars. These cultivars are different in resistance to *D. coccus*. It was also observed that there is sharing of characteristics among species in relation to resistance to *D. coccus*. Three cultivars from different species are resistant to the insect.The resistance characteristics could be shared among species since there is a genetically interlinked among the *Opuntia* spp. During the two cycles experiments, 'Rojo Pelón' was found to be resistant against *D. coccus* among the *O. ficus-indica* cultivars, because the insects could not develop and complete the life cycle but died at the nymph I stage. This is the first report on *D. coccus* resistance, which can be considered as an element of integrated pest management intervention or practice in Tigray and other areas having similar problems. It can be used for direct utilization and/or genetic improvements for the *D. coccus* resistance breeding programs for beles. Mechanisms of resistance or characteristics of the plants related to *D. coccus* resistance are also important subjects of study to use as a selection/improvement marker in resistant breeding. And it could be physical barriers, tissue structures, and secondary metabolites or combinations which lead to antibiosis (restricting growth or killing) resistance. Differently from the other cultivars, moulting of the insect was restricted in the 'Bioplástico' cultivar, this could be probably due to presence of unique secondary metabolites, and these phenomena of resistance are an important area of study.

Ethics statement

Not applicable

Consent for publication

Not applicable

Disclosure statement

The authors declare that they have no conflict of interest.

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Author contributions

All authors participated in the conceptualization of the project, made the investigation for the background of the manuscript, and wrote part of the original draft. The second and third authors participated in the application and development of the experiments; executed part of the administration of the project. They reviewed and edited all versions of the manuscript.

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2.2 Prueba de genotipos de *Opuntia ficus-indica* para resistencia a *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae)

Berhe Y. K., Portillo L. y Bolaños M. V. 2023. Testing *Opuntia ficus-indica* genotypes for resistance against *Dactylopius coccus* Costa**.** *Revista Colombiana de Entomología*

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Prueba de genotipos de *Opuntia ficus-indica* (L) para resistencia a *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae)

Testing *Opuntia ficus-indica* (L.) genotypes for resistance against *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae)

Abstract: Besides being a beneficial insect when it is cultivated for its carminic acid, the cochineal insect (*Dactylopius coccus* Costa) is also a threat to the prickly pear cactus (*Opuntia ficus-indica*), a plant that is extremely important as a source of food, cattle feed, income, and soil and water conservation. In a greenhouse, a test for resistance of four cactus pear genotypes to cochineal insects was conducted at the University of Guadalajra, Center of Sciences for Biology and Agriculture. Three of these genotypes (‘Punto 1’, ‘Punto 2’, and ‘Sandate 2’) were shown to be non-hosts for *D. coccus*. These genotypes can be used for further research and development purposes of prickly pear cactus.

Keywords: Cochineal insect, scale insect, prickly pear cactus, resistant.

Resumen: Aparte de ser un insecto benéfico cuando se cultiva para la producción del ácido carmínico, la grana cochinilla (*Dactylopius coccus* Costa) también se considera como una amenaza para el nopal (*Opuntia ficus-indica*), una planta que es extremadamente importante como fuente de alimento, forraje para el ganado, ingresos y conservación del suelo y el agua. Se realizó una prueba de resistencia a la grana cochinilla con cuatro genotipos de nopal en un invernadero del Centro Universitario de Ciencias Biológicas y Agropecuarias de la Universidad de Guadalajara. Se demostró que tres de los genotipos ('Punto 1', 'Punto 2' y 'Sandate 2') no son huéspedes de *D. coccus*. Estos genotipos se pueden utilizar para fines de investigación y desarrollo posteriores.

Palabras clave: Grana cochinilla, insecto escama, nopal, resistencia.

Introduction

Opuntia ficus-indica (L), known as cactus pear, is a multipurpose plant of great significance due to its ability to tolerate high temperatures, dry conditions, and marginal areas (Barbera et al., 1992; Berhe and Siyum, 2022). Stintzing and Carle (2005) characterized the prickly pear cactus as a "miracle plant, dromedary of the plant world and bank of life", and has greater agricultural importance in the semi-arid area of Tigray, Ethiopia (Gebremeskel et al., 2013; Berhe and Siyum, 2022). Almost all rural communities in eastern and southern Tigray and some parts of central Tigray use cacti as a staple food for about four months and as a cash crop, covering 360,000 ha (Brutsch, 1997; Berhe and Siyum, 2022).

The cochineal insect, *Dactylopis coccus* Costa (Hemiptera: Coccomorpha: Dactylopiidae) is a prickly pear cactus insect, that reproduces sexually; the female has 2 nymphal instars before reaching the adult stage, whereas the males go through 2 feeding nymphal instars, and non-feeding prepupal and pupal stages before reaching the adult stage which has a pair of wings (Pacheco da Silva et al., 2022). *Dactylopius* spp. are often considered beneficial for producing a red dye and also used for controlling weedy cacti (especially in South Africa and Australia), but are considered as pests when they attack cultivated cacti (Kondo, 2022). *D. coccus* has been reared commercially as a source of cochineal dye for centuries and is broadly utilized as a colorant in foods, including drinks, cosmetics, and medicinal products, and to dye textiles; the dried body of adult females produced in Bolivia, Chile, Mexico, Peru, and Spain (Pacheco da Silva et al., 2022). Damage caused by *D. coccus* and other species of the genus *Dactylopius* includes chlorosis (yellowing of the cladodes), dehydration and weakening of the host, and dieback of the plants in 6–10 months (Pacheco da Silva et al., 2022). On several continents, *Dactylopius* species pose a threat to prickly pear cactus. *D. coccus* was reported to damage large areas planted with prickly pear cactus in Tigray in Ethiopia (Belay, 2015; Berhe et al., 2020), and *D. opuntiae* is a significant pest that has been rapidly spreading over several nations, especially in the Mediterranean basin (Bouharroud et al., 2016; Kondo, 2022; Mazzeo et al., 2019). Prickly pear cactus production suffers severe losses because of the *D. opuntiae* epidemic in Brazil's semiarid region (Pacheco da Silva et al., 2022; Torres & Giorgi, 2018). The pest consequences of *D. coccus* in Tigray, Ethiopia, are socio-economic losses such as income reduction and youth migrations (Berhe and Siyum, 2022). It is also registered as pest in Chile (IPPC, 2010).

The development and utilization of resistant varieties is a vital constituent of integrated pest management strategy to control *D. coccus* (Berhe et al. 2020; Primo, 2020; Sabbahi & Hock, 2022). Tovar et al. (2005) found low *D. coccus* performance in certain varieties. Since *D. coccus* had not established itself, Méndez-Gallegos et al. (2010) suspected some form of resistance. Thus, the selection of cochineal-resistant prickly pear cactus genotypes is important for the mitigation of the pest problem (Berhe et al., 2020).

Materials and methods

A greenhouse experiment was executed, at the Center of Biology andAgriculture Sciences (CUCBA) of Guadalajara University in Mexico from December 2021 to April 2022 in a greenhouse. Four genotypes were tested: 'Sandate 2, 'Punto 1', and 'Punto 2'; and one susceptible host ('Chicomostoc'). These genotypes were selected based on field observations and information obtained from growers. Design of the experiment was completely randomized with three replications (cladodes as an experimental unit). The plants were grown in pots and cladodes for each plant were selected to be inoculated, while they were attached to their mother plant. 'Punto 1', and 'Punto 2' were grown from seeds while the others were vegetatively propagated. Adult females of *D. coccus* were reared in separate cladodes and were prepared for inoculation. The adult females were removed from their original host cladodes with a fine brush and kept in small paper bags. The insects were inoculated by fixing the paper bags with spines holding 20 mature females each. The paper bags remained attached to the cladodes for seven days allowing the ovipositing and infestation of the cactus pear cladodes (Figure 1 a-d) (Vigueras et al., 2005; Gusqui Mata, 2013; Berhe et al., 2022).

The number of first-instar nymphs after one week of infestation, and the number of adult females at maturity (100 days after infestation), were counted. The number of first-instar nymphs was counted seven days after infestation, while the number of adult females was counted when they started oviposition. The data were subjected to descriptive statistical analysis and average values were calculated.

Results

All genotypes had first-instar nymphs (crawlers) on the 7th day of infestation with a number ranging from 180 to 350 (Table 1 and Figure 1a-d). The nymphs reached maturity at the host genotype ('Chicomostoc') and 224 individuals were counted (Table 1 and Figure 1f-h), remained at first-instar stage at 'Punto 1', the whole life cycle period, but died at 'Punto 2' and 'Sandate 2' genotypes (Figures 1b and 1c). Similar screening works for *Dactylopius* spp. resistance has been reported before. Kuti (1990) identified five resistant *Opuntia* genotypes, while Batista et al. (2022) screened four *D. opuntia* genotypes as resistant out of 121 tested genotypes. Berhe et al. (2022) also found one resistant *O. ficus-indica* cultivar against *D. coccus*. The genotypes may be resistant through several mechanisms/strategies, both chemical and physical (Akroud et al., 2021). Being in the first-instar stage at accession 'Punto 1' is an indication of *Opuntia*'s variable resistance mechanisms (Musengi et al., 2021).

Table 1. Average± Standard error (SE) number of nymphs and adult female insects in the genotypes tested.

Accession	Average number of nymphs	Average number of female adults	Remark
Chicomostoc	350±10.48	224±8.33	Susceptible/control
Punto 1	200±16.83	0	Resistant
Punto 2	250±10.41	0	Resistant
Sandate 2	180±13.84	0	Resistant

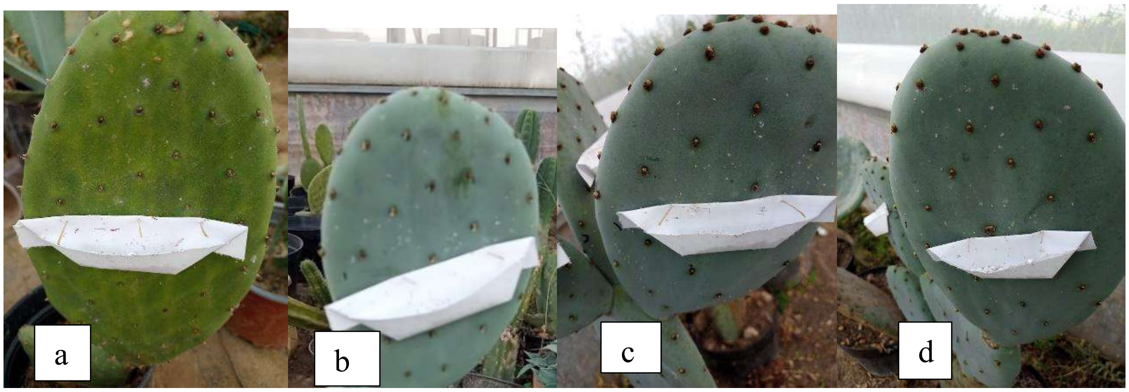

Figure 1. Cochineal insect survival and development under *Opuntia ficus-indica* genotypes tested after 7 days of inoculation, white spots are observed that show the presence of cochineal insects (a, Chicomostoc; b, Punto 1; c, Punto 2; and d Sandate 2), and after the completion of the insect life cycle period (e, Chicomostoc, a susceptible host with cochineal insects that completed the life cycle; f, Punto 1 (resistant) the insects could not develop beyond the first-instar stage; g, Punto 2 (resistant) without cochineal insects; and h, Sandate 2 (resistant) without cochineal insects.

Conclusion and recommendations

The evaluated genotypes of *O. ficus-indica* cultivars, namely ‘Chicomostoc’, ‘Punto 1’, ‘Punto 2’, and ‘Sandate 2’ showed different responses to *D. coccus*. All genotypes except ‘Chicomostoc’ were found resistant to *D. coccus*, since insects could not survive and developed to maturity. At ‘Punto 1’ insects remained at the first-instar stage. These resistant genotypes can be used as part of *D. coccus* integrated management in areas where this insect is considered a pest. They can be also used for genetic improvements and other research purposes. Chemical and physical features of the genotypes associated with cochineal defense mechanisms are an imperative subject of research.

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CAPÍTULO 3. OXALATO DE CALCIO Y OTRAS CARACTERÍSTICAS DEL CLADODIO EN CULTIVARES DE *Opuntia ficus-indica* RESISTENTES A *Dactylopius coccus* COSTA

Berhe, Y.K., Portillo, L., Delgado-Aceves, L., Palacios-Juárez, H. y Vigueras, A. 2023. Calcium Oxalate and Other Cladode Features in *Opuntia ficus-indica* Resistant Cultivars to *Dactylopius coccus* Costa. *Journal of Agricultural Chemistry and Environment*, 12, 112-123. doi: 10.4236/jacen.2023.122009.

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Calcium Oxalate and Other Cladode Features in *Opuntia ficus-indica* Resistant Cultivars to *Dactylopius coccus* Costa

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Abstract

Multipurpose cactus pear plant with great potential as a source of food and livestock faced a threat from *Dactylopius* spp in different countries. Specifically, *D. coccus* is an important pest damaging significant areas in Tigray-Ethiopia. Using pest-resistant cultivars is an important element of an integrated pest management strategy, and studying the mechanisms of resistance is vital. It can be chemical or physical such as oxalate crystals and other cladode characteristics. Cladode features of six cultivars (three *O. ficus-indica*, two *O. cochenillifera*, and one *O. robusta*) were examined for resistance to *D. coccus* in a completely randomized design (CRD) with three replications. 'Rojo Pelón' (*O. ficus-indica*), 'Robusta' (*O. robusta*), and 'Bioplástico' (*O. cochenillifera*) are resistant cultivars; and 'Atlixco' and 'Chicomostoc' (*O. ficus-indica*) and 'Nopalea' (*O. cochenillifera*) are susceptible. Cultivars showed a significant difference in cladode weight in g, and cladode length, cladode width, and cladode thickness in cm, where cladode thickness was higher in 'Rojo Pelón' followed by 'Robusta'. Calcium oxalates number per mm was higher in 'Bioplástico' (20.7 ± 2.08) followed by 'Robusta' (18.9 ± 2.31) and 'Rojo Pelón' (15.9 ± 0.34); and similarly, epidermis thickness found higher in 'Bioplástico' (0.21 ± 0.032) and 'Robusta' (0.19 ± 0.014), but similar with 'Rojo Pelón' (0.18 ± 0.026). However, cuticle thickness didn't show a difference among cultivars. Cladode thickness, calcium oxalate number, and epidermis thickness had positive correlations with resistance. These results demonstrate that calcium oxalate number and epidermis thickness might have a positive role in

D. coccus resistance in *O. ficus-indica*. This feeding-barring role and the insect-plant interaction need to be studied.

Keywords

Cactus Pear, Resistance, Druses, Epidermis Thickness

1. Introduction

Cactus pear (*Opuntia ficus-indica)* has many uses and huge potential mainly as human food and forage, its advantages include good biomass yield, it can grow on marginal land, being year-round evergreen, tolerant to drought, and crop during difficult times [1] [2]. It also gives better yield in suitable lands [3]. However, it is threatened by *Dactylopius* species on different continents. *D. coccus* damaged 75,000 ha in Tigray, Ethiopia [4] [5] and *D. opuntiae* is a devastating pest in Brazil and the Mediterranean region [6] [7] [8].

The development and use of resistant varieties are advantageous for the economy, ecology, and environment [9]. Understanding the levels and mechanisms of resistance is paramount for integrated pest management strategies [10] [11]. There are some resistant cultivars of *O. ficus-indica* to *D. coccus*. [12] and [13] reported differences in yield among *Opuntia* varieties in Mexico and discussed that resistance may occur. [14] identified the cultivar 'Rojo Pelón' (*O. ficus-indica*) resistant to this insect.

Mechanisms of resistance could be due to phytochemicals [11] or mechanical barriers such as calcium oxalate crystals and histological structures [15]. Higher concentration of these crystals may make it difficult for nymphs to insert their stylets and settle on the cladodes [16].

Naturally formed mineral crystals in plants can serve as a very effective insect defense [16] [17]. Calcium oxalate can be a physical barrier against chewing insects by an abrasive effect that blunts insects' mandibles and may act as an anti-nutritive defense by decreasing the efficiency with which ingested food is digested [18] [19]; so, it may deter the feeding of beetles [20]. Soluble oxalate or oxalic acid protects plants from herbivory by sucking insects [21] [22] [23]. However, the protective role of calcium oxalates can be observed in certain plants and need trial confirmation [24]. The presence of calcium crystals in different *Opuntia* spp was reported [25] [26] and the accumulation level varies depending on cultivars, growth stage, and other agronomic conditions [27] [28].

The cactus pear stem has a thick cuticle (13 - 20 μm) and a mono-stratified epidermis (117.33 - 120.07 μm) containing calcium crystals [29] [30]. Oxalate crystal size increases as a function of maturation [29] [31] [32]. Engineered calcium oxalate crystal is suggested to confer insect resistance [15]; it affects *Bamisiatabacai* feeding choice [33]. Both oxalate crystals and oxalic acid inhibit the sucking of brown planthoppers [22]. Calcium oxalates can affect digestion and harm the mouthparts of insects [24] [34] [35]. *Opuntia* cultivars have different sizes of cuticle and epidermis [36] [37] that could also be a barrier to *Dactylopius* spp [8] [12] [38]. From the above pieces of evidence, it can be the hypothesis that calcium oxalate and other cladode features may have a role in *D. coccus* resistance of *O. ficus-indica* cultivars. Thus, this study was conducted to investigate the role of calcium oxalate, epidermis thickness, cuticle thickness, and other cladode features in *O. ficus-indica* resistant cultivars to *D. coccus*.

2. Materials and Methods

2.1. Experimental Design and Treatments

Experimental analysis was carried out at the Biotechnology Laboratory of the Botany and Zoology Department of the University Center of Biological and Agricultural Sciences of Guadalajara University in 2020. The experiment consisted of six cultivars, which are three *O. ficus-indica* (resistant 'Rojo Pelón' and susceptible 'Atlixco' and 'Chicomostoc'), two *O. cochenillifera* (resistant 'Bioplástico' and susceptible 'Nopalea') and one *O. robusta* (resistant 'Robusta'), arranged in a completely randomized design (CRD) with three replications. These cultivars were chosen based on their resistance or susceptibility to the insect *D. coccus* *[14]*. Cladodes of the study cultivars were collected from several production farms in different locations of Mexico (Guadalajara, Jalisco; Ojuelos, Jalisco; and Villanueva, Zacatecas). Matured, vigorous, and free of plagues and diseases cladodes were selected for taking samples. Cladode length and width were measured with a ruler; thickness was measured with Vernier caliper, and weighing balance was used to measure cladode weight.

2.2. Sample Preparation Examining

Samples were taken from the middle part of the cladodes, which consisted of embedding the samples in polyethylene glycol (PEG) 1450 M mass in a 1:4 proportion (PEG: deionized water) according to [39], with a modification that boiled samples instead of fresh ones. A rotatory microtome was used to obtain 15 μm sections from the samples in PEG; then, they were stained with a double treatment using safranin 0.5% (1:1 w/v) and 0.5% toluidine blue (1:1 w/v). A light microscope (with a magnification level of 10X) was used to analyze the tissues. Anatomical data; cuticle thickness, epidermis thickness, and oxalate crystals number were recorded. Measurement of the epidermis (EP), respective cuticles (CU), and oxalates number per 1 mm length were performed using the software ImageJ.

2.3. Data Analysis

The results were subject to Analysis of variance (ANOVA) and correlation with statistical software package R.4.2.0. Least significant difference (LSD) test at (0.05) was applied to compare means among treatments. Paired t-test was also done to compare resistant and susceptible groups. The association of cladode characteristics and insect establishment was done using Pearson's correlation coefficient.

3. Results and Discussion

3.1. Cladode Characteristics of Different Cultivars of *Opuntia*

The cultivars showed a very highly significant difference in cladode weight (g) ($P = 0.0034$), width (cm) ($P = 0.0033$), and thickness (cm) ($P = 0.0000$); and a significant difference in cladode length (cm) ($P = 0.0104$) (**Table 1**). In line with this finding, differences in cladode morphological characteristics (width, length, and thickness) of *Opuntia* spp [40]) and *O. ficus-indica* cultivars have been reported [41].

3.2. Anatomical Characteristics of Different Cultivars of *Opuntia*

Means of epidermis thickness and number of calcium oxalate showed very highly statistically different ($P = 0.0000$), but cuticle thickness did not show a statistically significant difference ($P = 0.3660$). 'Bioplástico' followed by 'Robusta' and 'Rojo Pelón' showed the highest number of calcium oxalate crystals. Similarly, 'Bioplástico' followed by 'Robusta' and 'Rojo Pelón', demonstrated higher epidermis thickness than the other cultivars (**Table 2**).

Table 1. Means ± SE of cladode weight (CWt) in g, length (CL), width (CW), and thickness (CT) in cm of six *Opuntia* cultivars.

Cultivar	Resistance	CWt	CL	CW	CT
Rojo Pelón	R	1260.00 ± 174.74^{c}	36.00 ± 3.78^{bc}	17.3 ± 1.20^{bc}	3.50 ± 0.14^{d}
Atlixco	S	863.00 ± 220.48^{abc}	39.00 ± 2.67^{c}	19.7 ± 3.18^{c}	2.20 ± 0.14^{b}
Chicomostoc	S	986.67 ± 114.06^{c}	38.67 ± 2.60^{c}	18.0 ± 1.16^{c}	2.33 ± 0.17^{bc}
Bioplástico	R	420.00 ± 52.99^{bc}	33.00 ± 2.64^{bc}	11.8 ± 0.44^{ab}	2.42 ± 0.08^{bc}
Nopalea	S	190.00 ± 52.92^{a}	28.67 ± 2.31^{ab}	9.7 ± 0.34^{a}	1.59 ± 0.08^{a}
Robusta	R	900.00 ± 208.16^{c}	24.67 ± 1.85^{a}	23.0 ± 30^{c}	2.67 ± 0.08^{c}

Means sharing the same letter are not significantly different at $P \leq 0.05$. SE Standard error; R-resistant; and S-susceptible.

Table 2. Means ± SE results of epidermis thickness, cuticle thickness, and calcium oxalates number of six *Opuntia* cultivars.

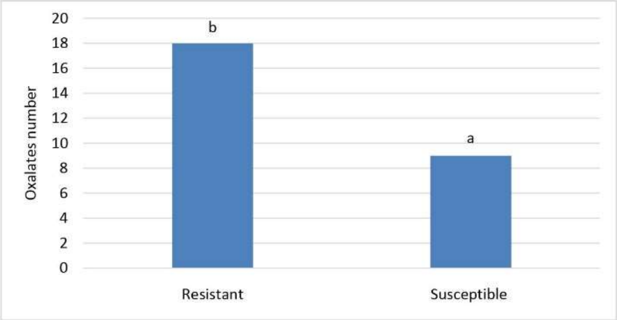
Cultivar	Oxalate number	Epidermis thickness	Cuticle thickness
Rojo Pelón	15.9 ± 0.34^{b}	0.18 ± 0.026^{abc}	0.0293
Atlixco	9.0 ± 0.23^{a}	0.13 ± 0.006^{a}	0.0223
Chicomostoc	9.3 ± 0.88^{a}	0.14 ± 0.010^{ab}	0.0470
Bioplástico	20.7 ± 2.08^{c}	0.21 ± 0.032^{c}	0.0281
Nopalea	8.9 ± 0.66^{a}	0.13 ± 0.007^{a}	0.0230
Robusta	18.9 ± 2.31^{bc}	0.19 ± 0.014^{bc}	0.0233

Means sharing the same letter are not significantly different at $P \leq 0.05$. SE Standard error.

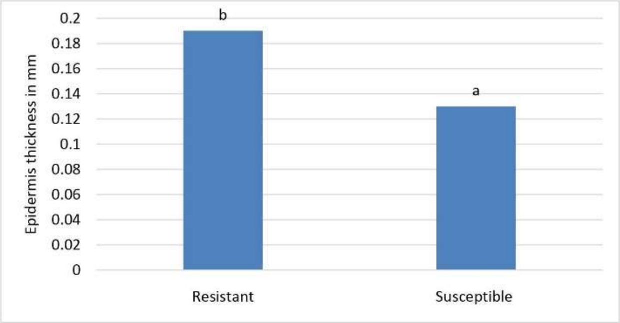
The resistant groups showed significant differences and double the number of calcium oxalate (18) than the susceptible group which scored 9 (P = 0.0000), and the average epidermis thickness of the resistant and susceptible cultivars was 0.19 mm and 0.13 mm respectively, with significant differences (P = 0.0015) (**Figure 1**).

The calcium oxalates observed are circular crystals (druse) [38]. Differences in the density of calcium oxalate crystals were reported among 15 cactus pear cultivated species of *Opuntia*, which ranged from 18 up to 57 per mm^2 [16]. The layer of calcium oxalates hampers the insertion of the stylets of *D. opuntiae* into the host plant [8]. Calcium oxalate crystals have negative effects on the growth of *Spodoptera exigua*, and the larvae also prefer to feed on *Medicago truncatula* lacking calcium oxalate [42]. They are also toxic to insects including sub-sucking [23] [24] [43] [44] [45]. This is because calcium oxalate crystals seem to serve as a feeding deterrent to insects [15] [19] [35]. It reduces growth rate and increases insect mortality; and hampers ingestion [35] [43] [46].

The difference in epidermis thickness is also supported by previous similar



(a)



(b)

Figure 1. Chart showing the average number of oxalate crystals (a) and epidermis thickness in mm (b) of the resistant and the susceptible *Opuntia* cultivars to *Dactylopius coccus*.

research. The difference in epidermis thickness is also supported by previous similar research. 2 - 3 μm width of the cuticle, 6 to 10 μm width of the epidermis, and difference among *Opuntia* spp were observed [36]. [16] mentioned that the epidermis was the main anatomical barrier to *D. opuntiae*, providing greater resistance and integrity of the cladode and varying among studied cacti. The author cleared that those cacti with thick epidermis are insect resistant. The thickness of the epidermis and cuticle can be good resistance factors of *Opuntia* spp to the cochineal (*D. opuntiae*) [38].

From **Figure 2**, it can be depicted that calcium oxalate crystals seen as spots are denser at the resistant cultivars (a) to (c) than at the susceptible cultivars (d) to (f). Pearson's correlation coefficient analysis showed that cladode thickness (0.68), calcium oxalate number (0.89), and epidermis thickness (0.75) have a significant positive correlation with *D. coccus* resistance (**Table 3**).

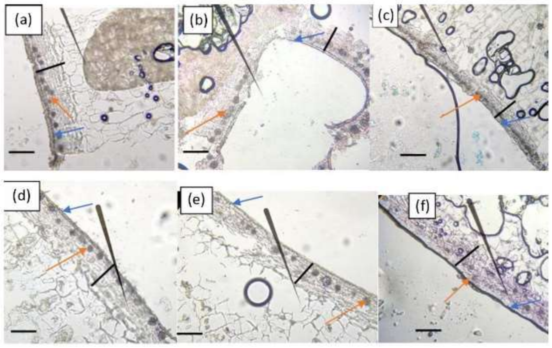


Figure 2. Microscopic view of histological cuts of cladodes showing calcium oxalates (indicated with red arrow), the epidermis (indicated with black solid line), and cuticle (indicated with blue arrow) of six *Opuntia* cultivars, (a) 'Rojo Pelón', (b) 'Robusta', (c) 'Bioplástico', (d) 'Atlixco', (e) 'Chicomostoc', and (f) 'Nopalea' detected with a microscope at a magnification level of 10X. Bars = 110 μm.

Table 3. Pearson correlation among different morpho-anatomy of cladodes of six *Opuntia* cultivars and *Dactylopius coccus resistance*.

	R	CL	CW	CT	CwT	OxN	CuT	EpT
R	1	−0.35	0.15	0.68*	0.22	0.89*	−0.13	0.75*
CL		1	0.23	0.19	0.51*	0.33	0.27	−0.08
CW			1	0.47*	0.80*	0.06	0.03	0.48
CT				1	0.76*	0.44	−0.03	0.48*
CwT					1	0.02	0.16	0.09
OxN						1	−0.07	0.77*
CuT							1	−0.08
EpT								1

*Significant at P ≤ 0.05 probability level. R-resistance; CL-Cladode length; CW-Cladode width; CT-Cladode thickness; CWt-Cladode weight; OxN-Oxalates number; CuT-Cuticle thickness, and EpT-Epidermis thickness.

In this research, the calcium oxalate crystals and epidermis thickness in cladodes of *O. ficus-indica* appeared to have a strong association with resistance against *D. coccus*. Similar previous research done by [47] on sap-sucking insects affirmed these findings. Active host-plant resistance mechanisms against aphids involve resistance factors based at, or within, the leaf epidermis or within the phloem sap, such as defense chemistry, reduced nutritional content, and lower palatability [48]. Other authors also discussed that resistant mechanisms of *Panicum virgatum* (Poaceae) against *Sipha flava* (Aphididae) could reduce amino acid content and higher oxalic acid levels. Calcium oxalate is the precipitation form of oxalic acid [49] and increased concentrations of this acid promote calcium oxalate crystals formation [50].

Due to the reduction of nutritional value [51], the presence of these crystals in plants is unfavorable for herbivores and has negative effects on sucking insects [24] [43] [52]. Calcium oxalate crystals may be also sufficient to have some detrimental effects on insect fitness by damaging their mouthparts [24]. [16] stated that a high concentration of calcium oxalate crystals prevents nymphs from inserting their stylets and establishing themselves on the cladodes. [53] concluded that in conifer stems the patterns and frequency of calcium oxalate crystals function as a constitutive defense and in combination with fiber rows, provide an effective barrier against small bark-boring insects. An increased number of calcium oxalate enhanced insect resistance in *Prunus avium* cultivars [54]. Orchids recruited the strongest defensive strategies, consisting of a thick epidermis, a larger proportion of needle-like calcium oxalate crystals, and higher content of alkaloids and quinones [55], indicating that plants can use one or different physicochemical defense mechanisms [56].

In this study 'Rojo Pelón', an *O. ficus-indica* and *D. coccus* resistant cultivar found with a greater number of calcium oxalate crystals, originated from Northern Guanajuato and Southern San Luis Potosi in the Central Highlands of Mexico [57] [58]. This cultivar is a vigorous plant with sweet, bright red, and export-quality fruits and for having spineless cladodes it is useful as forage [58]. It could be a good candidate as an alternative to those regions in the world including Tigray, Ethiopia, where resistant *Opuntia* cultivars to *D. coccus* are needed.

4. Conclusion

The number of calcium oxalate crystals (druses) and epidermis thickness was found to be higher in resistant cultivars and positively correlated with resistance as in previous works also confirming that the number of oxalates and epidermis thickness can contribute to insect resistance either by deterring or toxifying the insect. Further study could be important to assess other resistant contributing factors and the plant-insect reaction during the feeding process. Using resistant varieties is an important integrated pest management strategy for the environment and for the producers.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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CAPÍTULO 4. METABOLITOS SECUNDARIOS EN *Opuntia ficus-indica* RESISTANTES A *Dactylopius coccus* COSTA

4.1 Análisis fitoquímico de cultivares de *Opuntia ficus-indica* resistentes a *Dactylopius coccus* Costa

Phytochemical analysis of *Opuntia ficus-indica* cultivars resistant to *Dactylopius coccus* Costa

Phytochemical analysis of *Opuntia ficus-indica*cultivars resistant to *Dactypopius coccus* Costa *Dactylopius coccus* Costa

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Abstract

Dactylopius coccus (cochineal) is an important pest of *Opuntia ficus-indica* of *Opuntia ficus-indica.* Several *Opuntia* cultivars are resistant to cochineal, but the underlying resistance mechanisms are unknown. Objective of this research is to investigate the phytochemical difference among *O. ficus-indica* cultivars that might involve in *Opuntia*-cochineal interactions. The host preference of cochineal was evaluated using six *Opuntia* cultivars (‘Atlixco’, ‘Nopalea’, ‘Chicomostoc’, ‘Rojo Pelón’ ‘Bioplástico’ and ‘Robusta’). Cochineal completed its life cycle in ‘Atlixco’, ‘Chicomostoc’, and ‘Nopalea’ cultivars but not in the remaining cultivars. Moreover, we evaluated the cultivars' hydrogen peroxide, protein, polyphenol, and flavonoid contents after cochineal infestation (herbivore damaged (HD)). The levels of total protein content were increased in the HD cultivars, ‘Robusta’ and ‘Bioplástico’. In addition, a significant positive correlation was recorded between the number of nymphs with total proteins and flavonoids and the number of adults with flavonoids, which supports that these chemicals might be associated with the susceptibility or suitability of the *Opuntia* cultivars.

Key words: Plant-insect interactions, cochineal, cactus pear cultivars, plant metabolites

Introduction

Dactylopius spp (Hemiptera: Dactylopiidae) and commonly known as cochineals, are a important pest of cactus pear, *Opuntia ficus-indica* (Berhe et al. 2020, Bouharroud et al. 2016, Mazzeo et al. 2019). For instance, *D. coccus* damaged significant areas of cactus pear plantations in Tigray, Ethiopia (Belay 2015, Berhe, et al. 2020), and *D. opuntie* threatened the cactus pear in Morocco (Bouharroud et al. 2016). *Dactylopius* species have piercing-sucking mouthparts, allowing them to feed on different plant cells. Hence, *D coccus* can have a significant effects on cactus pear production (Chávez-Moreno et al. 2009).

Control of cochineal depends mainly on pesticides. The most used chemical pesticides in producing cactus pear cactus include but are not limited to neonicotinoids, organophosphates, carbamate, and pyrethroids. However, these pesticides cause undesirable damage to human, animal and environment. Resistant varieties can be utilized to mitigate these problems. *D. opuntiae* resistant varieties were reported, but the fundamental causes of resistance are not disclosed. In Brazil, in laboratory and field tests, some *Opuntia* varieties observed to be resitant against *D. opuntiae* (Borges et al. 2013, Passos da Silva et al. 2009, Vasconcelos et al. 2009)

Secondary metabolites widely involve in plant insect interactions (War et al. 2012). Studies indicated *O. ficus-indica* is rich in natural antioxidant compounds (Avila-Nava et al. 2014, El-Hawary et al. 2020, Nassrallah et al. 2021, Saleem et al. 2006). Some secondary metabolites found can be defensive against *D. coccus* (López-Palacios and Peña-Valdivia 2020). *O. ficus-indica* genotypes have shown different levels of defensive compounds (Farag et al. 2020, López-Palacios and Peña-Valdivia 2020).

Total polyphenols and flavonoids increase the antioxidant capacities of cacti (Silva-Beltrán et al. 2015). Flavonoids are used in plant defense against biotic and abiotic factors (Panche et al. 2016). Different flavonoid levels present in plants affect insect behavior differentially (Khalid et al. 2019). Flavonoids found in soybean Glycine max (L.) Merr. (Fabaceae) distorted insect herbivores' metabolism, behavior, and development (Stec et al. 2021). Lu et al. (2017) reported that the total flavonoid content reduced damaged leaf area in rust-resistant *Malus* plants.

Proteins in plants are also important nutrition content for herbivores, and the total protein content is essential to insects (Le Gall and Behmer 2014). An assortment of nutrients are consumed by insect herbivores to support the processes of growth, development., and reproduction (Behmer, 2009). Following the detecting of insect herbivory damage signals, plants have a unique early reaction that involves the rapid generation of superoxide radicals and formation of hydrogen peroxide (H_2O_2). (Maffei et al. 2007, Zebelo and Maffei 2014). H_2O_2 was induced in response to soybean cyst nematode (SCN) infection (Chen et al. 2020). By inducing the synthesis of flavonoid polymers in plant cells, it improves rice and other important cereals' resistance to sap-sucking insects. (Wang et al. 2020).

Winter triticale (*Triticosecale Wittm*) produces secondary metabolites like phenolic acids when they are damaged by grain aphid (*Sitobion avenae*) (Chrzanowski and Leszczyński 2008). Flavonoid (3-deoxyanthocynadin) inflicted death on corn leaf aphid (*Rhopalosiphum maidis*) in sorghum (Kariyat et al.,2019). Likewise, the growth and fitness of adult tobacco hornworm, *Manduca sexta* L negatively impacted polyphenol-rich maize varieties (Tayal et al. 2020). Polyphenols used by many plant species against a wide range of insect pests (Tayal et al. 2020).

Limited studies assessed the role of secondary cactus metabolites in their interaction with cochineal. This research is intended to investigate the phytochemical difference among *O. ficus-indica* cultivars, explicitly focusing on the content of hydrogen peroxide, total protein, total polyphenols, and flavonoids with potential ramifications in *O. ficus-indica*-cochineal interactions.

Materials and Methods

Plant material and sample preparation

The research included of six *Opuntia* cultivars. 'Rojo Pelón', 'Atlixco', and 'Chicomostoc', 'Nopalea','Bioplástico'), and 'Robusta′ (Table 1). ‘Atlixco’, ‘Nopalea’, and 'Chicomostoc’ cultivars were susceptible to cochineal, and ‘Rojo Pelón’, ‘Bioplástico’, and ‘Robusta’ cultivars were resistant to cochineal (Berhe et al., 2022). The field-collected cladodes were thoroughly washed with clean tap water. The matured, vigorous, and diseases and insect-damage-free cladodes from each cultivar were used for the experiment. Completely randomized design (CRD) was applied. There were two treatments (Control (undamaged) and Herbivore Damaged (HD)), each treatment consisted of four biological replicates, totalling eight cladodes per cultivar, and the four replicates were used as a control, and the remaining four used for HD. The control and HD cladodes were numbered and marked with their respective treatment and replicate

Insect Infestation

Adult *D. coccus* females reared in separate cladodes were prepared for the HD cladodes. First, the 20 adult females were collected and saved in paper bags. Next, the paper bags containing 20 adult females each were hooked with spines of their respective replicates and cultivars. According to Roque-Rodríguez (2022), the paper bags stayed attached for a week. Then, after approximately 100 days, the insect's life cycle is completed on the susceptible cultivars. However, in the resistant cultivars, the insects were not able to complete their lifecycles. Insect counts per cladode were recorded, and samples (cladodes) from the control and HD cladodes were collected into liquid nitrogen using a punching instrument and after that stored at -80°C. Fresh-frozen samples were then lyophilized and homogenized. Finally, finely powdered plant tissue was weighed in polypropylene microfuge tubes and used for the following analysis.

Total cladode protein content determination

Total protein content in control and HD cladodes was quantified following Huseynova (2012) methods. The control and HD cladode samples were freeze-dried and homogenized in a mortar. The 0.1 g sample was weighted using a sensitive Mettler balance and added to 5 mL of pre-chilled 0.1 M phosphate buffer under ice-cold conditions. The homogenized extract was centrifuged at 17600 g for 20 min. Bradford Assay was used to quantify the total leaf protein content with some modification i.e., 280 µL of Bradford reagent was added to 15 µL of enzymatic extracts. It was allowed to incubate for 10 min before it was analyzed with a plate reader (BioTek™ Synergy 2 BioTek).

Hydrogen peroxide content

Following the method used by Lara-nuñez et al. (2006), we quantified the level of Hydrogen peroxide content in control and HD cladode samples. The cladode samples were homogenized, and 50 mg of the homogenized samples were added to 750 µL of 50 mM phosphate buffer at pH 7 and mixed and vortexed. The mixture was centrifuged at 6000g for

20 mins, and 600µL supernatant was carefully removed and added to 200 µL of 0.1% titanium tetrachloride in 20% v/v H_2SO_4. The mixed solution was centrifuged at 6000 g for 15 min, and absorbance was determined at 415 nm using a plate reader (Thermo Scientific™). The hydrogen peroxide concentration was computed using 0.28 μmol^{-1} cm^{-1} as the extinction coefficient (Omagamre et al., 2022).

Total polyphenols and flavonoid content

To quantify the total polyphenols and flavonoid content levels, 50 mg of lyophilized cladode samples were homogenized with 1mL of 50:50% acetonitrile/water. The mix was sonicated for 10 min at room temperature. Then, the sonicated solution was centrifuged at 8000g for 10 mins, and the extracts were collected into Eppendorf tubes (Omagamre et al., 2022). With little modifications, the total phenolic content of cladode samples was quantified following the method by Singleton et al. (1999). In 96-well microplates, 15 µL cladode extracts from the different samples were added to 240 µL of distilled water. To the mixture, 15 µL of Folin-Ciocalteu reagent was added and incubated for 3 min, and the reaction was stopped with 30 µL of 4N Na_2CO_3. This final mix was stayed at room temperature for 2 hours, and after that, the absorbance at 760 nm was measured using a microplate reader (Biotek Synergy, NJ USA). A calibration standard was developed using different levels of gallic acid to quantify the total phenol content, and the result was reported as mg gallic acid equivalents (GAE)/g dry weight.

Following Ghasemi et al. (2009), the total flavonoid content was quantified. The method involved mixing 20 µL of cladode extract from different samples, 112 µL of distilled water, 60 µL of 80% methanol, 4 µL of 0.5 M aluminum trichloride solution, and 4 µL of 1 M potassium acetate in a 96-well plate. After incubating at room temperature in the dark for 30 mins, absorbance was measured at 415 nm. As a result, the total flavonoid content was reported as mg quercetin equivalents (QE)/g dry weight (Omagamre et al., 2022).

Results

Cochineal prefers susceptible cultivars

Variations among cultivars were significant in the number of nymphs, (p=0.0000) and adults (p=0.0005) of *D. coccus* (Figure 1). A minor quantity of nymphs was found on 'Rojo Pelón', followed by 'Robusta', and superior quantity on 'Nopalea', followed by 'Atlixco' (Figure 1). Cochineal failed to complete its lifecycle in the resistant cultivars 'Rojo Pelón', 'Robusta', and 'Bioplástico' (Figures 1 and 2). However, cochineal could grow, colonize, and complete its life cycle at susceptible cultivars. As a result, the average number of adult cochineals per cladode in 'Atlixco', 'Chicomostoc', and 'Nopalea' cultivars was 32, 25, and 3, respectively (Figures 1 and Figure 2).

Herbivory by cochineal reduces the protein content of Opuntia cultivars

Protein content significantly differed among the control cultivars (p=0.0245) but did not differ substantially on the herbivore-damaged cultivars (p=0.843). The 'Nopalea' cultivar showed the highest protein content, followed by the 'Atlixco' cultivar. These cultivars are both susceptible to *D. coccus*. At the same time, fewer protein levels were recorded in the 'Bioplástico'(resistant) and 'Chicomostoc'(susceptible) cultivars (Figure 3). Interestingly, after *cochineal infestation,* the levels of total protein content were increased in the HD-resistant cultivars, 'Robusta'and 'Bioplástico' and decreased in the HD-susceptible cultivars, 'Atlixco' and 'Nopalea' (Figure 3).

The levels of Hydrogen peroxide (H_2O_2) in cochineal-infested Opuntia cultivars

The level of H_2O_2 was lower in control susceptible cultivars, 'Atlixco' and Nopelea, and the level of H_2O_2 was higher in cochineal damaged (HD) 'Atlixco' and 'Nopelea' cultivars (Figure 4). On the contrary, the level of H_2O_2 was higher in the resistant control cultivars, 'Bioplástico' and 'Rojo Pelón', and the level of H_2O_2 decreased after cochineal infestation. However, there was no significant difference among cultivars in hydrogen peroxide content between the control and HD samples in susceptible and resistant cultivars (p=0.0617 and 0.198, respectively) (Figure 4).

The total phenolic acid content flavonoid contents in cochineal-infested Opuntia cultivars

Total polyphenols content at both conditions (Control and HD) and flavonoid content on HD showed very high statistical differences among cultivars (P=0.000), while flavonoids content on control cultivars showed significantly different (P=0.0428). Polyphenolic content was higher in control ‘Rojo Pelón’, ‘Chicomostoc’, and ‘Atlixco’ cultivars than in the other three control cultivars. After infestation, ‘Chicomostoc’showed higher polyphenols, followed by ‘Rojo Pelón’. ‘Atlixco’recorded higher flavonoid content before infestation, similar to ‘Chicomostoc’ (Table 2). In most cultivars, the polyphenols and flavonoid content levels were reduced after cochineal infestation, except in the ‘Chicomostoc’cultivar. However, there were no significant differences in the polyphenols and flavonoid content levels after cochineal infestation within the ‘Atlixco’, ‘Chicomostoc’, and ‘Robusta cultivars. There was a substantial reduction in polyphenol content in ‘Bioplástico’ (P=0.020) and ‘Nopalea’ (P=0.023) cultivars after cochineal infestation. Interestingly, the level of polyphenol content increased significantly in the ‘Rojo Pelón’ (P=0.021) cultivar following cochineal infestation.

Cochineal population correlates with the phytochemical contents of Opuntia cultivars

The average number of cochineal nymphs increases with the protein contents of both the susceptible and resistant *Opuntia* cultivars, except with the ‘Rojo Pelón cultivar, where the number of cochineal nymphs decreases with the increase of the protein contents (Figure 5). Similar trends were recorded with the level of flavonoids in *Opuntia* cultivars, where the average number of cochineal nymphs increases with the rise of the flavonoid’s contents of both the susceptible and resistant *Opuntia* cultivars, except with the ‘Rojo Pelón’ cultivar, where the number of cochineal nymphs decreases with the increase of the flavonoids contents (Figure 6). Interestingly, a similar trend was recorded in the correlation of the number of cochineal nymphs versus the polyphenol levels. The average number of cochineal nymphs increases with the levels of polyphenol of the susceptible and resistant *Opuntia* cultivars, except with the ‘Robusta cultivar, where the number of cochineal nymphs decreases with the increase of the polyphenol contents (Figure 7). The number of nymphs with total proteins and flavonoids and the number of adults with flavonoids were positively correlated (Table

3), which supports that these chemicals are associated with the susceptibility or suitability of the *Opuntia* cultivars.

Discussion

The acceptability of the host plant by the insect herbivory depends on the development stage and physiological condition (Schoonhoven et al. 2005), previous experience (Turlings et al. 1993) of the insects, and morphological and physiological conditions of the host plant(Schoonhoven, et al. 2005). Host acceptability and suitability of cactus cladodes to *D. coccus* nymphs depend on whether the neonates pierce and suck sap from the cactus cladodes. Insects' success in colonizing a plant host is assessed by their ability to contribute a viable number of offspring to the next generation. In the present study, we evaluated the preference of *D. coccus* (cochineal) for *O. ficus-indica (*'Atlixco', 'Chicomostoc', and 'Rojo Pelón', cultivars)*, O. cochenillifera* ('Bioplástico and 'Nopalea', cultivar)*, and O. 'Robusta'* ('Robusta', cultivar). Even though the cultivars belong to the same species, some are susceptible, and others are resistant. For instance, 'Atlixco', 'Chicomostoc', and 'Rojo Pelón' cultivars belong to *O. ficus-indica,* but 'Atlixco' and 'Chicomostoc' cultivars are susceptible, and 'Rojo Pelón' is resistant to *D. coccus.* Cochineal failed to complete its lifecycle in the resistant cultivars 'Rojo Pelón', 'Robusta', and 'Bioplástico'. However, cochineal grew, colonized, and completed its life cycle at susceptible cultivars such as 'Atlixco', 'Chicomostoc', and 'Nopalea'. Consistent with our findings, Musengi et al. (2021) reported that *O. stricta* and *O. engelmannii* are a morphologically similar taxon of *Opuntia*. However, cochineal was able to complete its lifecycle in *O. stricta*, but not in *O. engelmannii*. Despite *Opuntia* cultivars' morphological similarity in a given taxon, their cochineal susceptibility varies significantly. Here in this study, we demonstrated that the selective behavior of cochineal could be partly due to the secondary metabolite of the opuntia cultivars.

In Cochineal-infested resistant cultivars, 'Robusta' and 'Bioplástico'*,* the levels of total protein content were higher than the susceptible cultivars, 'Atlixco', and 'Nopalea'. Variations in the quality and quantity of protein content widely affect plant-insect interactions. Plants synthesize a various range of proteins that mediate defense against insect herbivores, comprising toxic, anti-nutritive, and anti-digestive proteins that interfere with the growth and development of insect herbivores(Glawe et al., 2003). For instance, a group of anti-digestive proteins is called protease inhibitors (PIs) (Green and Ryan 1972). Plants with high concentrations of PIs are resistant to insect herbivores (Jongsma and Bolter 1997). Glawe, et al. (2003) reported thatherbivores prefer and thrive more on *Nicotiana attenuata* genotypes with lower PI concentrations than genotypes with greater PI contents.. Broadway and Duffey (1988) concluded that protein value significantly alters the toxicity of soybean trypsin inhibitor to the larvae of.

Similarly, Chiozza et al. (2010) observed amino acids could be linked with nutritive value and could be vital in defining host fitness. Protein quality can be varied by genetics and environment and considerably influences herbivore performance (Felton 1996, Lu et al. 2007, Scheirs et al. 2003) and reproduction (Casteel et al. 2014, Scheirs, et al. 2003). We have seen the number of cochineal nymphs increase with the protein contents of both the susceptible and resistant *Opuntia* cultivars, except with the 'Rojo Pelón' (resistant) cultivar, where the number of cochineal nymphs decreases with the increase of the protein contents. The *Opuntia* cultivar used in this study might contain some protein that interferes with the growth and development of cochineal, so further investigation might be needed to study the type of protein involved in the susceptibility and resistance of the *Opuntia* cultivars.

The level of hydrogen peroxide (H_2O_2) increases after cochineal infestation in the susceptible cultivars ('Atlixco' and 'Nopalea') and decreases in the resistant cultivars ('Rojo Pelón' and 'Bioplástico'). Interestingly, following cochineal infestation, the level of H_2O_2 increased, and the total protein content decreased in susceptible cultivars. However, it was to the contrary in the resistant cultivars. Hydrogen peroxide is one of the reactive oxygen species (ROS). It has many functions in plants, and one of the functions is that it serves as an early plant defense signalling that triggers downstream signalling events such as the production of phytohormones that initiate the gene expression of defense genes that regulate the production of defensive proteins and other secondary metabolites (Zebelo and Maffei 2014). Insect infestation induces peroxidase activity in sap and plant leaves' total soluble protein (TSP) (Singh et al. 2013). A positive correlation was found between H_2O_2 content and pod insect damage that suggested the accumulation of H_2O_2 in response to pod borer attack (Kaur et al. 2014). H_2O_2 actively interfere with insects' digestive system, leading to reduced growth and development (Mohamed et al. 2020, War et al. 2012, Zhu-salzman et al. 2005).

It was evident that the level of polyphenols and flavonoids varies among the cultivars used in this study. In general, higher levels of polyphenol and flavonoids were recorded in the *O. ficus-indica* cultivars, which contradicts the findings of Guevara-Figueroa et al. (2010), who reported the highest total phenolic acid content in the wild varieties of nopal than in the *O. ficus-indica* cultivars. Flavonoid content is more elevated at susceptible cultivars, but War et al. (2016) flavonoids serve for induced resistance against insect pests. In addition, Matos et al. (2021) reported some flavonoids in cactus pear species associated with resistance to carmine cochineal. These compounds can be toxic or deterrent to the insect or favourable to herbivores, depending on the insect's specific compound and sequestration ability (Lu et al. 2017, Panche et al. 2016, Simmonds 2001). The level of flavonoids was lower in the resistant cultivars and higher in the susceptible cultivars. This might be associated with the lower number of cochineal reported in the resistant cultivars. Goławska et al. (2008) observed that pea aphid performance was lower at alfalfa with lower flavonoid content. In other ways flavonoids can enhance host suitability for the insect (Simmonds 2001). Comparing the cultivars of *O. ficus-indica* only, the arithmetic mean shows that 'Rojo Pelón' has higher total polyphenols in the control samples. 'Chicomostoc' showed higher polyphenols content after infestation. Previous literature supports the higher content of polyphenols in resistant cultivars. Kaur et al. (2022) reported total polyphenols increased in *Chilo partellus* tolerant maize genotypes after infestation. The high level of polyphenols and low level of flavonoids can be related to the trade-off among secondary metabolites (Cipollini and Levey 1997, Stark et al. 2015, Treutter 2006).

In this study, a significant positive correlation was recorded between the number of nymphs with total proteins and flavonoids and the number of adults with flavonoids, which supports that these chemicals are associated with the susceptibility or suitability of the *Opuntia* cultivars. The protein content was higher at the susceptible cultivars, which may be related to nutrition suitability for the insect. Hydrogen peroxide may not have a direct role in the resistance mechanism of cultivars, but it may involve in triggering the cascade of plant defense signaling. Total polyphenols are superior in resistant cultivars, which indicates the level of total polyphenols is associated with resistance. Flavonoid content seems to stimulate and favor the *D. coccus* herbivory on *Opuntia*. Further research might be needed to investigate the type of protein, polyphenol, and flavonoid associated with the susceptibility and resistibility of the *Opuntia* cultivars to ratify the *Dactylopius-Opuntia* cultivar interactions.

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Figure legends

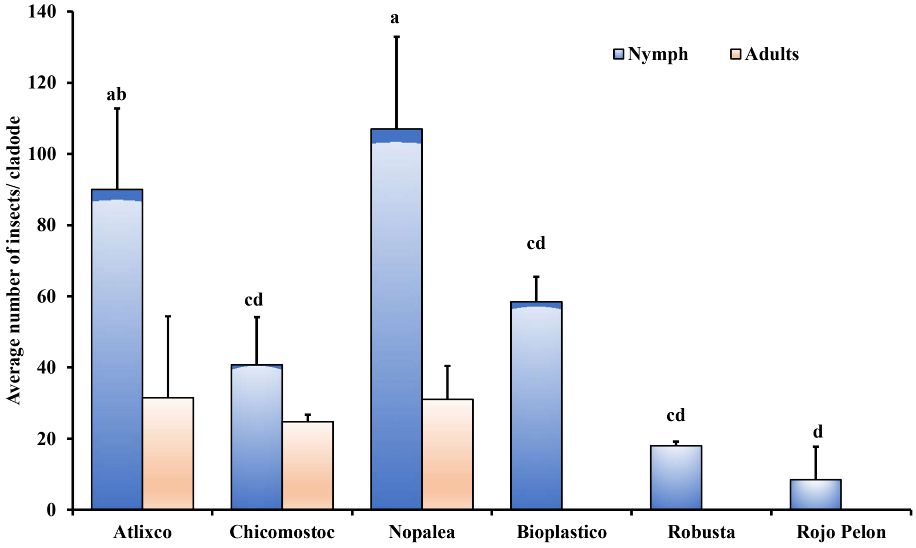

Figure 1. The average number of nymphs and adults of *Dactylopius coccus* on six *Opuntia* cultivars. Bars with different letters indicate a significant difference ($p<0.05$, $n=4$).

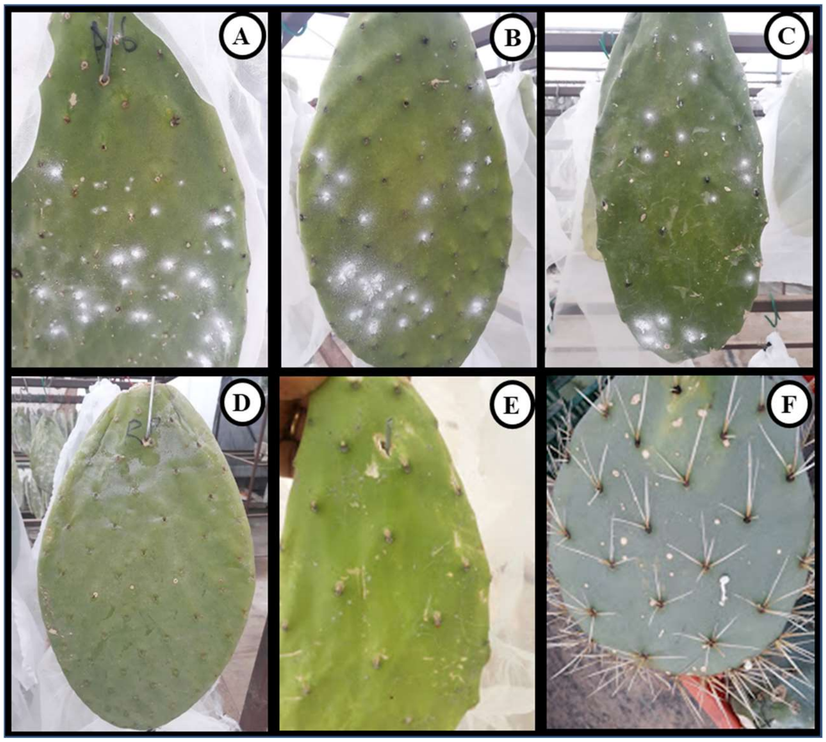

Figure 2. The photo depicts the six *Opuntia* cultivars used for the study and the abundance of cochineal (bright white spots). Three of the cultivars are susceptible, which have insects developed, '‘Atlixco’ '(A), '‘Chicomostoc’ ' (B), and '‘Nopalea’ '(C); and three resistant, which have no insect developed, 'Rojo Pelón' (D), '‘Bioplástico’ '(E) and '‘Robusta’ '(F)

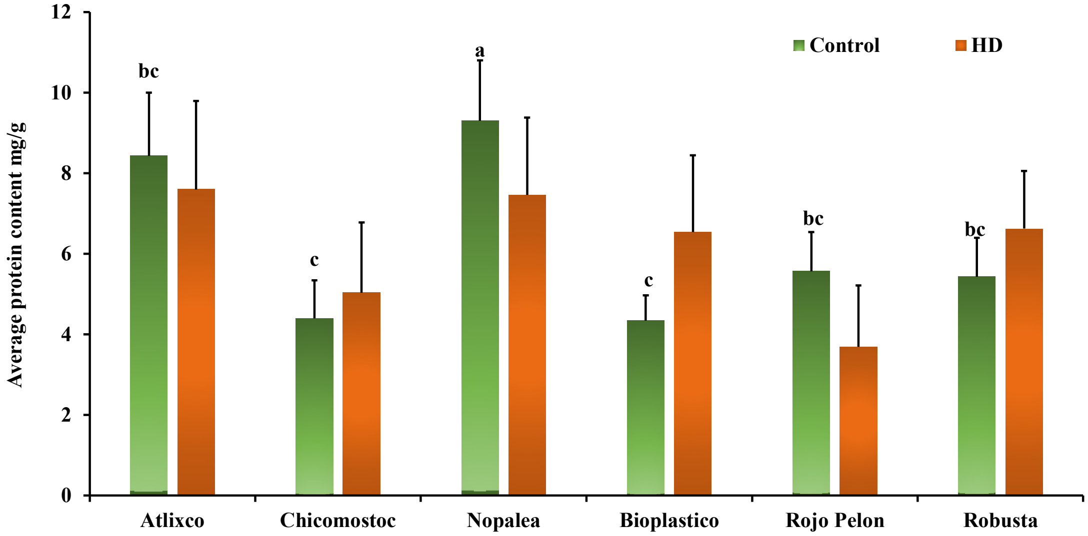

Figure 3. Protein content (mg/g) of different Opuntia cultivars. Bars with different letters indicate a significant difference between control and herbivore-damaged (HD) samples ($p<0.05$, n=4).

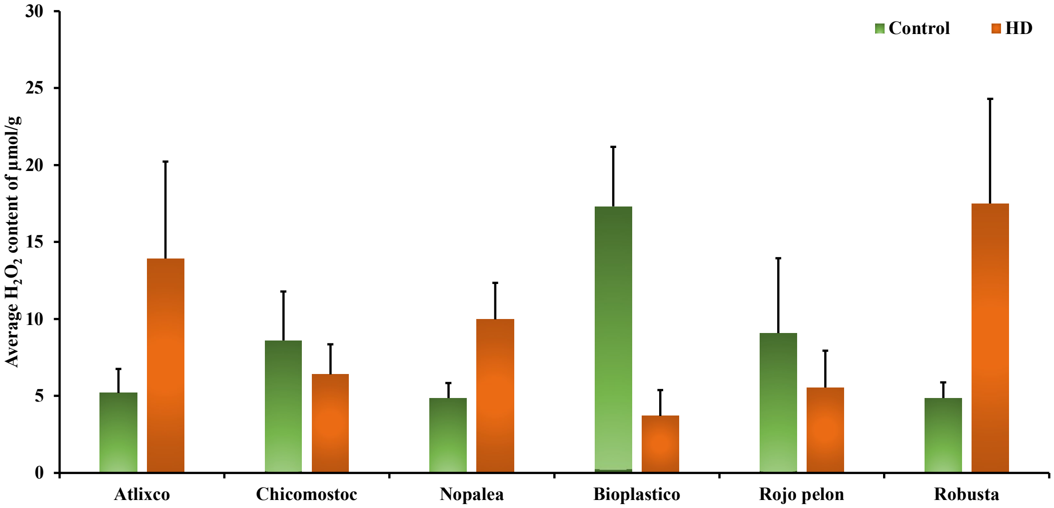

Figure 4. The levels of Hydrogen peroxide (H_2O_2) in cochineal-infested Opuntia cultivars. Bars show the levels of H_2O_2 in micromoles per gram of fresh weight in control and herbivore-damaged (HD) samples.

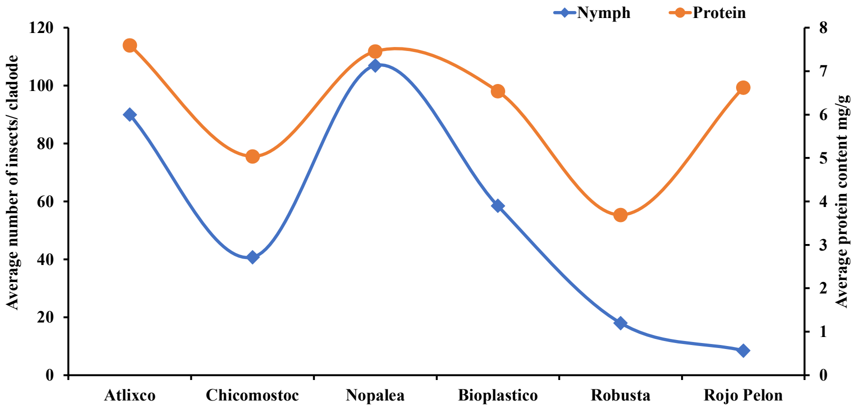

Figure 5. The correlation between the number of cochineal nymphs and the total protein content(mg/g) on six *Opuntia* cultivars.

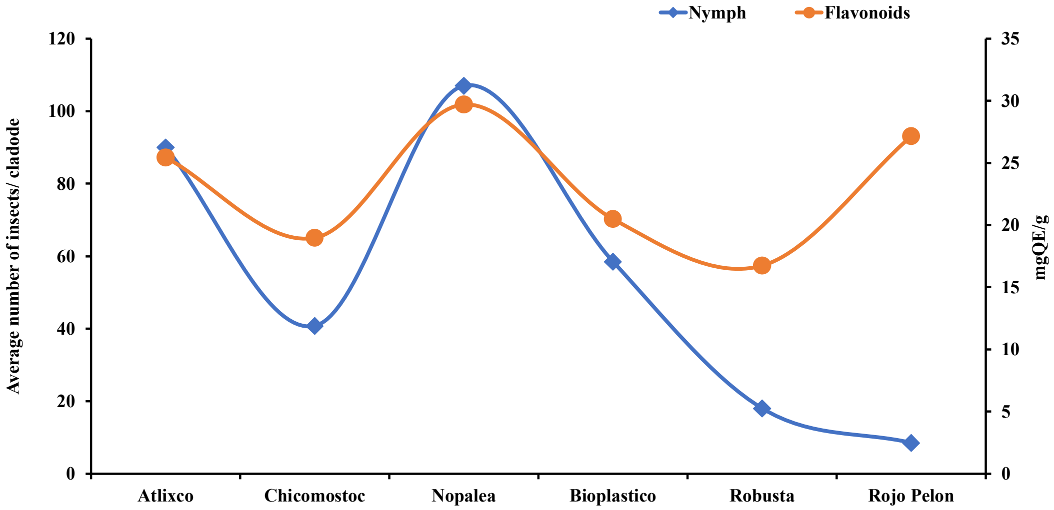

Figure 6. The correlation between the number of cochineal nymphs and the total polyphenol content (quercetin equivalents (QE)/g DW) on six *Opuntia* cultivars.

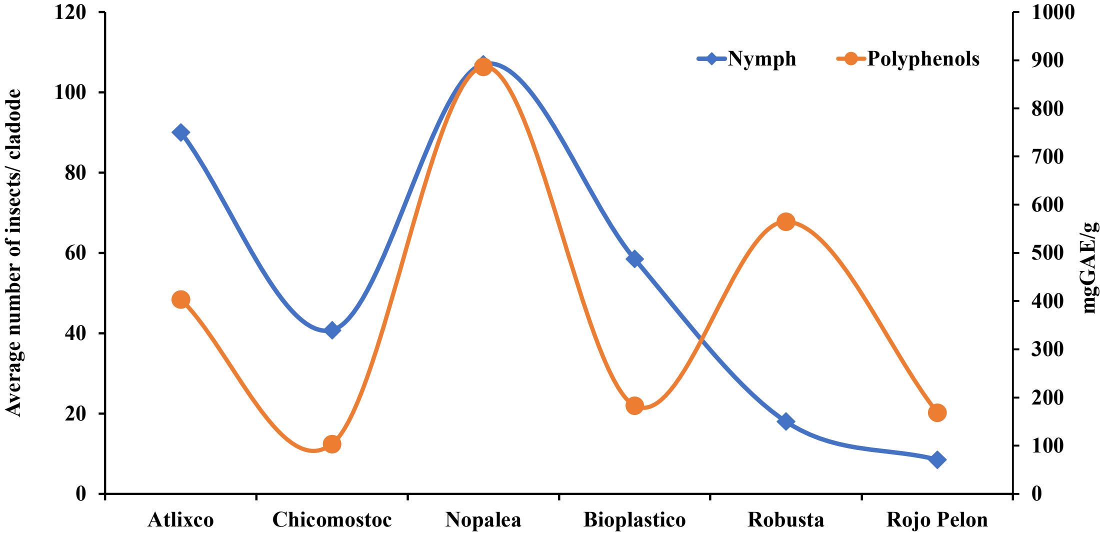

Figure 7. The correlation between the number of cochineal nymphs and the total flavonoid content (gallic acid equivalents (GAE)/g DW) on six *Opuntia* cultivars.

Table 1. Identity and cladode characteristics (cultivar name, species name, national ID, source and resistance to *Dactylopius coccus*) of the six *Opuntia* cultivars evaluated.

Cultivar name	**Species name**	**National Id**	**Source**	**Resistance**
Atlixco	*O. ficus-indica*	NOP- 018-221104	VNS	S
Bioplástico	*O. cochenillifera*	NA	VNS	R
Chicomostoc	*O. ficus-indica*	NA	VNS	S
Nopalea	*O. cochenillifera*	NA	GDLJ	S
Rojo pelón	*O. ficus-indica*	NOP-022-221104	OjJ	R
Robusta	*O. robusta*	NOP-060-090617	OjJ	R

Note: OjJ- Ojuelos, Jalisco, VNZ- Villanueva, Zacatecas, GDLJ-Guadalajara, Jalisco; R-resistan, S-susceptible. Source: Catálogo-Nacional-de-Variedades-Vegetales (CNVV), 2020

Table 2. Total flavonoid content expressed in quercetin equivalent (QE) per gram dry weight and polyphenols of samples of six *Opuntia* cultivars. The data is presented as mean ± standard error. Different letters across the row indicate a significant difference at $p<0.05$, $n=4$. The overall significance level indicated as follows $p<0.001$(***), $p<0.01$(**), and p 0.05(*).

Cultivars	**Polyphenol**(mgQE/g)		**Flavonoid**(mgGAE/g)	
	Control	**HD**	**Control**	**HD**
Atlixco	662.54±14^{a}	403.22±37^{c}	56.19±12^{b}	25.45±3^{ab}
Bioplástico	244.45±32^{a}	103.25±6^{c}	25.96±2^{a}	18.99±1^{a}
Chicomostoc	719.72±86^{a}	886.23±54^{a}	38.28±7 ^{ab}	29.71±3^{ab}
Nopalea	191.63±26^{b}	183.10±13^{d}	30.12±2^{a}	20.50±4^{b}
Rojo pelón	752.88±47^{a}	564.89±45^{b}	30.57±2^{a}	16.75±3^{ab}
Robusta	260.33±20^{b}	168.44±25^{d}	31.52±3^{a}	27.16±5^{b}
	***	***	*	***

Table 3. The coefficients of correlation of some phytochemicals with the number of insects at two growth levels (nymph and adult). The significant level of the correlation is indicated as follows, $p<0.001$(***), $p<0.01$(**), $p< 0.05$(*), and not significant(^{ns}).

	Peroxide	**Protein**	**Flavonoid**	**Total polyphenols**
Nymph	-0.01009993^{ns}	0.5253646***	0.4517039*	-0.2527426^{ns}
Adults	-0.132282^{ns}	0.273826^{ns}	0.4648644*	0.1782655^{ns}

4.2 Perfil no dirigido de metabolitos secundarios en cultivares de *Opuntia ficus-indica* relacionados con la defensa contra *Dactylopius coccus* Costa

Berhe, Y.K., Meza-Canales, I.D, Trujillo-Pahua, V., Sánchez-Hernández, C.V., Burgos, A., Peniche-Pavía, H., Hilda Eréndira Ramos Aboites, H.RA, Soto, S., Winkler, R., and Portillo, L. Untargeted profiling of secondary metabolites in *Opuntia* cultivars for defense against *Dactylopius coccus* Costa. *Plant Bioinformatics Section, Frontiers in Plant Science*

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Untargeted profiling of secondary metabolites in *Opuntia* cultivars related to defense against *Dactylopius coccus* Costa

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Keywords: Cactus pear cultivation, DLI-ESI MS, metabolic profile, plant resistance, arid crop plants

Abstract

Cactus pear (*Opuntia ficus-indic*a) cultivation has an essential role in the livelihood of people in arid and semi-arid regions of the globe (Lahbouki et al., 2022). Similar to other crop plants, cactus pear suffers from various stressors, with cochineal (*Dactylopius spp.*) being one of the most important. In some regions, the domestic cochineal (*Dactylopius coccus*) mismanagement has accentuated this problem, affecting cactus pear productivity. Using resistant varieties is a valuable option to control cochineal. However, since resistance traits can vary between different varieties and environmental conditions, it is essential to understand the basis of their resistance traits to generate breeding strategies. Here we explore changes in the metabolic profiles of cladodes from resistants (*O. ficus-indica,* cv. 'Rojo Pelón', *O. cochenillifera* cv. Bioplástico, and *O. robusta* cv. 'Robusta') and susceptible (*O. ficus-indica*, cv. 'Atlixco', and 'Chicomostoc', and *O. cochenillifera* cv. 'Nopalea') varieties to cochineal infestation. Mature cladodes before and after insect infestation and young cladodes were sampled and analyzed by untargeted DIESI-mass spectrometry. We identified 376 different m/z features across all tested samples. Supervised classification method of partial least squares-discriminant analysis (PLS-DA) allowed us to distinguish 38 metabolic features specifically associated with resistance to cochineal in all cultivars. Interestingly, 14 metabolic features were presented in higher quantities in resistant cultivars before infestation, suggesting a constitutive baseline resistance among non-susceptible cultivars to cochineal infestation. We found 37 features that differed in abundance when comparing young and mature cladodes, and six metabolites had higher quantity in young cladodes of susceptible cultivars. Furthermore, seven metabolic features associated with resistance were shared among mature resistant cultivars and young cladodes of all cultivars analysed.

1. Introduction

Due to its resistance to drought and high temperatures and its adaptability to poor soils, *Opuntia* spp (a.k.a. cactus pear) has become of substantial importance in the lives and work of people living on arid and semi-arid lands where many obstacles challenge food production (Inglese et al., 2017), as well as an interesting crop alternative in areas projected to be affected by climate change with high temperatures and droughts (Nafzaoui et al., 2012; Mungá et al., 2019). Although originally from Mexico (Griffith, 2004), Cactus pear cultivation has expanded to many other regions around the globe (Barbera et al., 1992; Inglese, 2010). However, currently, cactus pear plantations are being threatened by *Dactylopius* spp (Hemiptera) in many countries, like Israel, Brazil, Morocco, Spain, and Ethiopia (Spodek et al., 2014; Bouharroud et al., 2016; Torres & Giorgi, 2018; Berhe et al., 2020; Mazzeo et al., 2019). Moreover, mismanagement of the domesticated cochineal (*D. coccus*) has accentuated the problem for many regions, significantly damaging vast areas of cactus pear cultivation (Belay, 2015; Berhe et al., 2020). In Ethiopia, after introducing *D. coccus* for carmine production in the last decade, it has spread to natural and cultivated areas, significantly decimating Cactus pear plantations used for food or fodder (Fitiwy et al., 2016; Hailu, 2020; Berhe et al., 2020; IPPC 2022). Addressing this requires an integrated control approach, including different methods and considering the importance of environment protection and biodiversity. One sound sustainable alternative to counteract the current scenario is using varieties resistant to *Dactylopius* spp (Akroud et al., 2021; Mazzeo et al., 2019; El Finti et al., 2022, Berhe et al., 2020). However, much more research is required to understand the essential traits that explain the resistance of Cactus pear varieties, which may assist both selection and management.

Resistant varieties of *O. ficus-indica*, the main cactus pear crop, and other *Opuntia* spp. species to *D. coccus* have been reported (Tovar and Pando-Moreno, 20110; Mendez-Gallegos et al., 2010; Tovar et al., 2005; Tovar-Puente et al., 20105; Berhe et al., 2022). However, the mechanisms of resistance, either morphological or biochemical, which could be associated with genetic makeup, still need to be addressed. Plants respond to diverse environmental enemies with a bewildering array of responses (Molano-Flores, 2001; Nakata, 2015; War et al., 2020). They use physical and chemical defenses that may be constitutive or induced, affecting the plant's tolerance or resistance (Agrawal and Karban, 2000; Lattanzio et al., 2006; Kaplan et al., 2008; Erbilgin, 2019). Phytochemical defensive molecules include alkaloids, anthraquinones, flavonoids, phenols, saponins, steroids, tannins, terpenes and others (Ali et al., 2017; Namukobe et al., 2011; Agidew, 2022; Rizwan et al., 2022).

Opuntias contain many bioactive compounds (Allai et al., 2017; 2020; Akroud et al., 2021). Andreu et al. (2018) found a higher total polyphenolic content in young cladodes, that may act as a defense mechanism since young cladodes may not have developed other defensive mechanisms (Ventura-Aguilar, *et al*, 2017; Figueroa-Perez et al., 2018; Wit and Fouche, 2021; Leon-Solano et al., 2021). Dávila-Aviña et al. (2018) reported significant differences in total polyphenol among nine cactus cultivars. Some flavonoids are cytotoxic and interact with other organisms' enzymes through complexation (Amawi et al., 2017; Kejik et al., 2021). They protect the plant against insect pests by influencing survival, development, and insect behavior. Avila-Niva et al. (2014) identified 24 phenolic compounds, including *quercetin*, kaempferol, and isorhamnetin flavonoid compounds in *O. ficus-indica* cladodes. Furthermore, López-Palacios and Pena-Valdivia (2020) observed a higher total abundance of hydroxycinnamic acids in cladodes of wild species resistant to *D. coccus*. The authors suggested that the production of some of these compounds was reduced since domesticated cultivars are used as hosts for the cochineal (*D. coccus*) culture. The authors measured caffeic, chlorogenic, ferulic, syringic, and vanillic acids, which participate in insect defense in many plants. Nevertheless, plants respond with a complex rearrangement of their metabolism, including different metabolic pathways which execute different strategic functions.

'Atlixco', 'Chicomostoc', and 'Nopalea' are *D. coccus* susceptible, while 'Bioplástico', 'Rojo pelón', and 'Robusta' are *D. coccus* resistant (Berhe et al., 2022). We explored the metabolic profile of these cultivars to identify secondary metabolic responses associated with *D. coccus* resistance. To determine resistance-associated secondary metabolites, we conducted untargeted metabolomic profiling on resistant and susceptible cactus pear cultivars non-infested and infested with *D. coccus*.

2. Materials and Methods

2.1 Design of the experiment and Biological Material

Six *Opuntia spp.* cultivars from three different species previously reported by us as resistant and susceptible to *D. coocus* were used (Berhe et al., 2022); 'Atlixco', 'Chicomostoc' and 'Rojo pelón' (all *Opuntia ficus-indica*), 'Nopalea' (*Opuntia cochenillifera*), and Bioplástico (*O. cochenillifera*) and 'Robusta' (*Opuntia robusta*). The plant materials were kindly provided by growers Carlos Davila ('Atlixco' and 'Chicomostoc') and Silvestre Ruiz López (Bioplástico) from plantations in Villanueva, Zacatecas, Mexico, Armando Esparza González ('Rojo pelón' and 'Robusta') from plantations in Ojuelos, Jalisco, Mexico and by Miguel Alcalá ('Nopalea') from plantations in Guadalajara, Jalisco Mexico. All cladodes were carefully selected, ensuring they were vigorous and free of any visible plague or disease.

Cochineal (*D. coccus*) was obtained from in-house colonies at the University Center of Biological and Agricultural Sciences of the University of Guadalajara, reared on *O. ficus indica* cv. 'Atlixco' cladodes, under greenhouse conditions, at ambient temperature and humidity (Temperature range 10 to 38.05 ºC and relative humidity range of 36.95 to 56.77%). Matured female insects were used for infestation, carefully harvested with a brush from mature cladodes.

The experiment was conducted in a completely randomized design. Samples of the cultivars were collected from not-infested and infested mature (c. 1y old) cladodes, and young shoots (1 month old) grown from the stalks collected for the different varieties at the greenhouse. Samples from the infested cladodes were taken after the insect completed its life cycle. Each treatment consisted of at least four biological replicates.

2.2 Sample Preparation and Extraction

After collection, cladodes were thoroughly washed with pure water. A subset of all varieties was propagated by minimal fractions as described and grown for 1 month until sampling. The other set was either infested, or not, with cochineal using a soft brush and hung on the zootechnical greenhouse for the duration of one full insect cycle, and sampled afterward.

Samples were collected from the central portion of the cladodes with a cork borer and were flash-frozen in liquid nitrogen and stored at -80ºC. Fresh-frozen plant tissues were pulverized, homogenized, and then lyophilized. Finely powdered plant tissue (10 mg) was weighed in 2 ml polypropylene microfuge tubes. Direct liquid injection electrospray (DLI-

ESI)-mass spectrometry (MS) method was used for the chamical anlaysis. Extraction was done by adding 1 mL pure methanol with ultrapure water (80:20 v/v) containing 0.1% formic acid. Samples were mixed and placed in an ultrasonic bath at maximum frequency for 15 min at 10 ºC followed by a 10,000 g centrifugation for 10 min at 4 ºC. Finally, extracts were filtered with 0.2 µm pore size filters and stored at -20 ºC temperature until MS analysis.

2.3 Profile and screening of secondary metabolites

A 100 µL of each sample was taken with a glass syringe for direct injection in the electrospray ionizer of the mass spectrometer (DIESI-MS), at a speed 10 µL / min. The voltages of the ionization source were adjusted to 3kV and of the cone to 30V in negative mode (Peniche-Pavia and Tiessen, 2020; Salem et al*.,* 2016). Raw data were converted to the standard .mzML using the ProteoWizard MS. The .mzML data were imported into the R (R Version 4.1), and feature matrix data was generated with the complete m/z and intensity values and then converted to *.CSV format using R (Version 4.1) (Peniche-Pavia and Tiessen, 2020).

2.4. Statistical analysis

Differences in metabolism between resistant and susceptible cultivars were analyzed by supervised Partial Least Squares-Discriminant Analysis (PLS-DA), and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA).

To evaluate differences in metabolic profiles among the *Opuntia* cultivars, test the infestation condition, and the age of cladodes, Unsupervised Pas Coefficient analysis (PCA) and supervised latent structure-discriminant analysis (PLS-DA) were implemented.

These analyses showed the group variability in a graphic projection and enabled the selection of the discriminating metabolites (Thévenot et al., 2015). The PLS-DA and OPLS-DA models were cross-validated through the quality parameters R2 and Q2. The highest discrimination variable importance projection (VIP≥1.5) and 10% of the total metabolites were taken. This analysis was used to identifyy the most influential metabolites for discrimination between groups (Triba et al., 2015). PLS-DA statistical methods were performed in free R Software (RStudio, Version 1.2.5001 2009-2019, Inc., with R-3.6.1) using Bios Manager (Morgan and Ramos 2019), ropls package (Thévenot et al., 2015), and graphics (RCore Team, Version 3.6.3; 2018; Trujillo-Pahua et al., 2021) libraries.

3 Results

Untargeted metabolomic analysis of all *Opuntia* spp cladode samples detected 376 features (Figure 1), 366 features shared among all conditions (infested, not-infested, and young cladodes), 4 present only between infested and young cladodes, and 6 on infested and not-infested but not found on young cladodes (Figure 1a). Among cultivars, 368 features were detected in all plants. Only 8 features were not found in at least one *Opuntia* cultivar, five mz features on *O. chicomostoc* Bioplastic (136.71, 454.83, 822.46, 824.63, 878.36), two on *O. robusta* (356.81, 626.65) and one on *O. ficus-indica* 'Rojo pelón' (455.76).

Unsupervised Classification PCA analysis of all metabolic data show a clear separation among the treatments, showing a greater contribution of the treatment condition in the arrangement of the metabolic profiles than of the different species and cultivars (Figure 1b). Moreover, the unsupervised classification of cultivars for each treatment showed an abstruse separation among cultivars (Figure 1c).

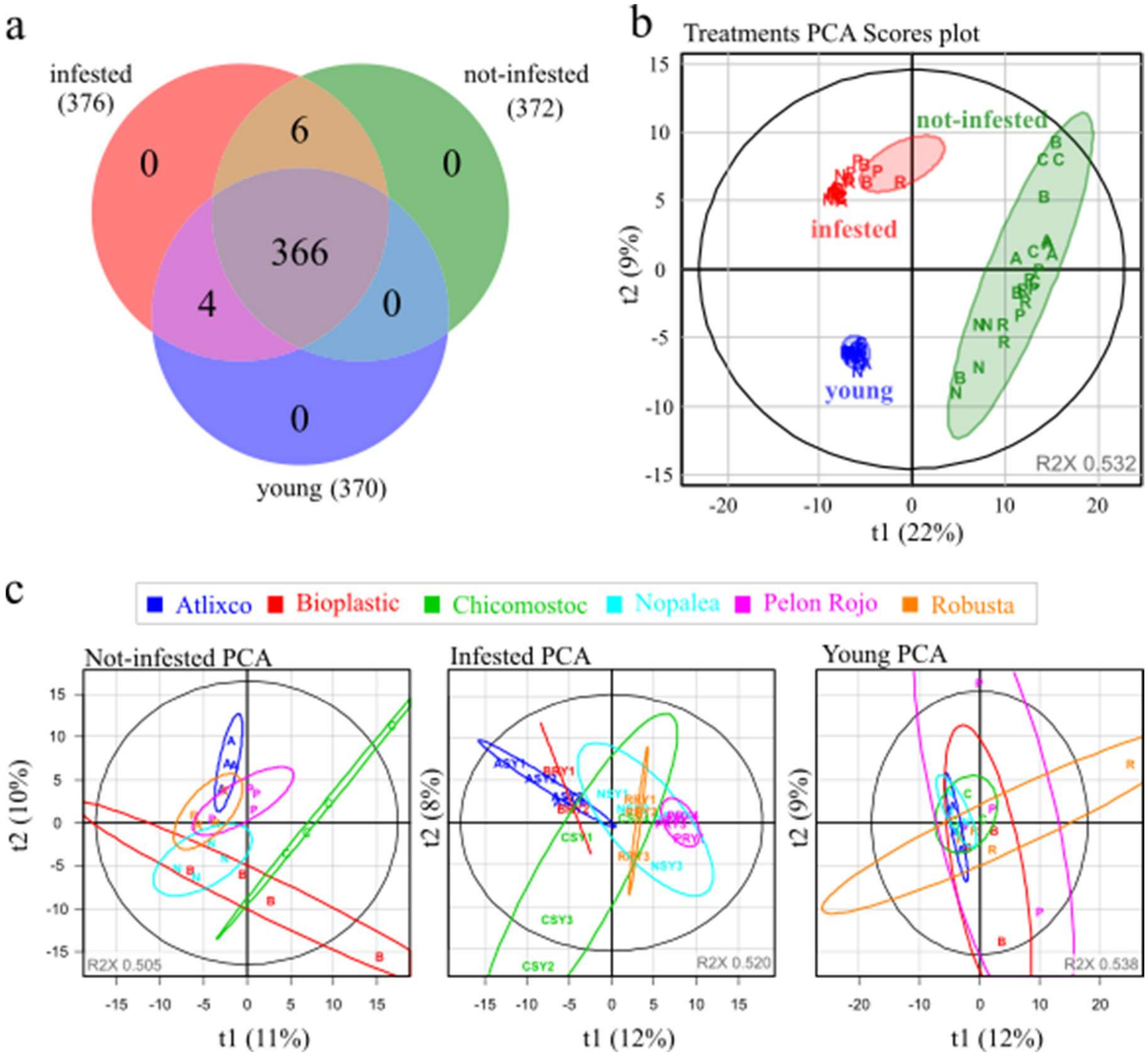


Figure 1. Venn Diagram, Unsupervised PCA classification. (a) number of metabolites by treatment group (non-infested, infested, and young cladodes), (b) classification of metabolites in different groups, (c) PCA score plots of cladode metabolites profile in cultivars *Opuntia* cultivars at non-infested, infested, and young cladodes.

3.1 Identification Metabolic Variations of Opuntia cultivars

Interestingly, PLS-DA results showed a clear separation between resistant and susceptible metabolic profiles from the cultivars analysed (Supplemental Figure 1). A clear distance between resistance and susceptible cultivars' metabolic profiles suggests that resistance to infection may be inherent to the basal secondary metabolism of cactus pear plants, prior to *D. coccus* infection.

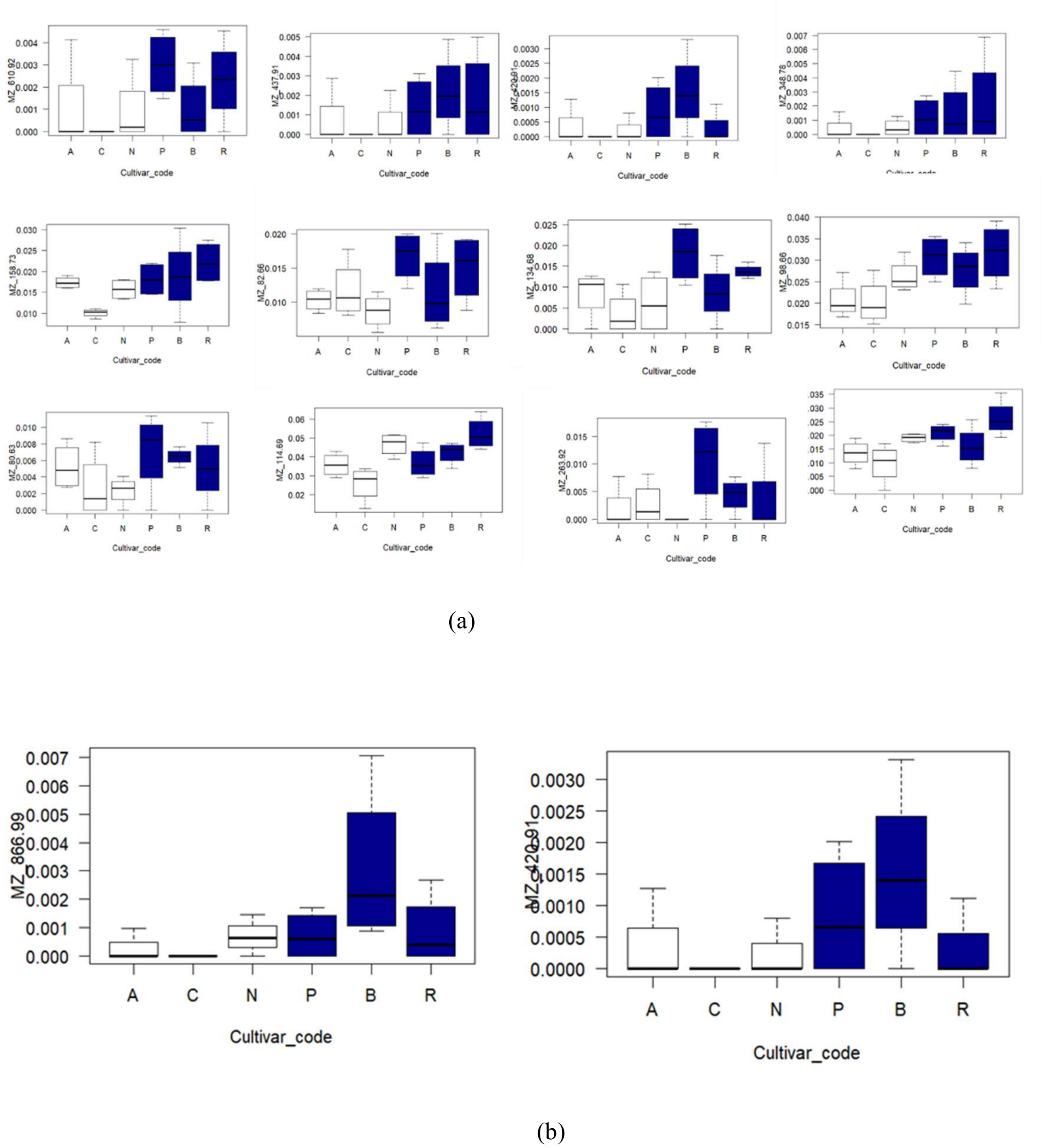


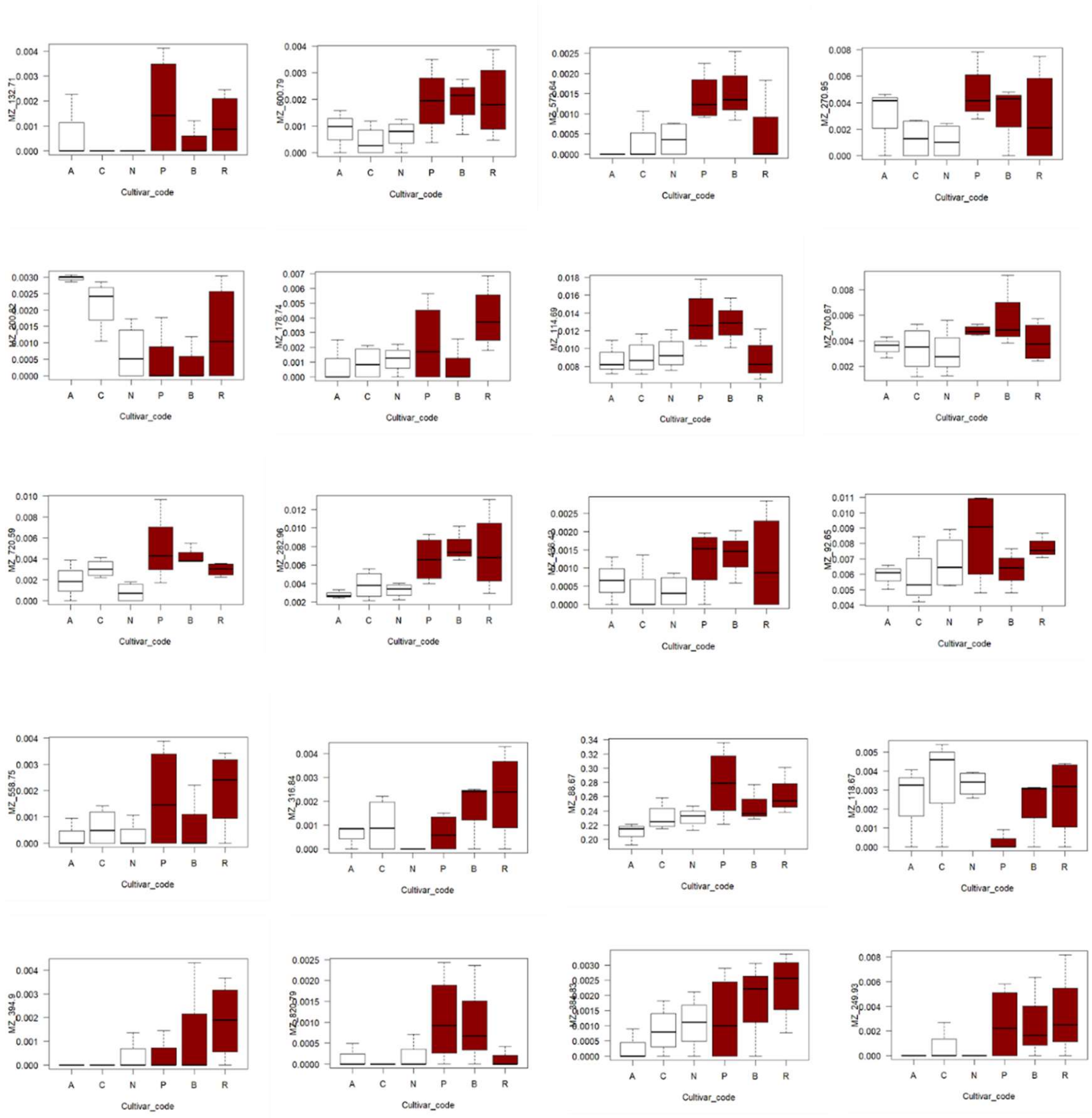
Figure 2. Relative abundance of selected insect resistance discriminant features (VIP≥1.5) presented in metabolic profile of *Opuntia* cultivars before infestation identified with PLS-DA. The box plots present summarized data of susceptible (white) and resistant (blue) cultivars. A, Atlixco; B, Bioplástico; C, Chicomostoc; N, Nopalea: P, Rojo Pelón; R, Robusta.

The presence of resistant related secondary metabolites with (VIP ≥ 1.5) is shown (Figure 2 and Supplemental Table 1). Out of the 324 secondary metabolites, 38 were found to discriminate susceptible and resistant cultivars non-infested, and 16 metabolites appeared with higher abundance in resistant cultivars. About 14 metabolite features like; mz_98.66, mz_158.7, and mz_348.7 appeared in higher concentrations in all resistant cultivars and mz_420.9 and mz_866.99 appeared in more concentrations in cv. ‘Bioplástico’.

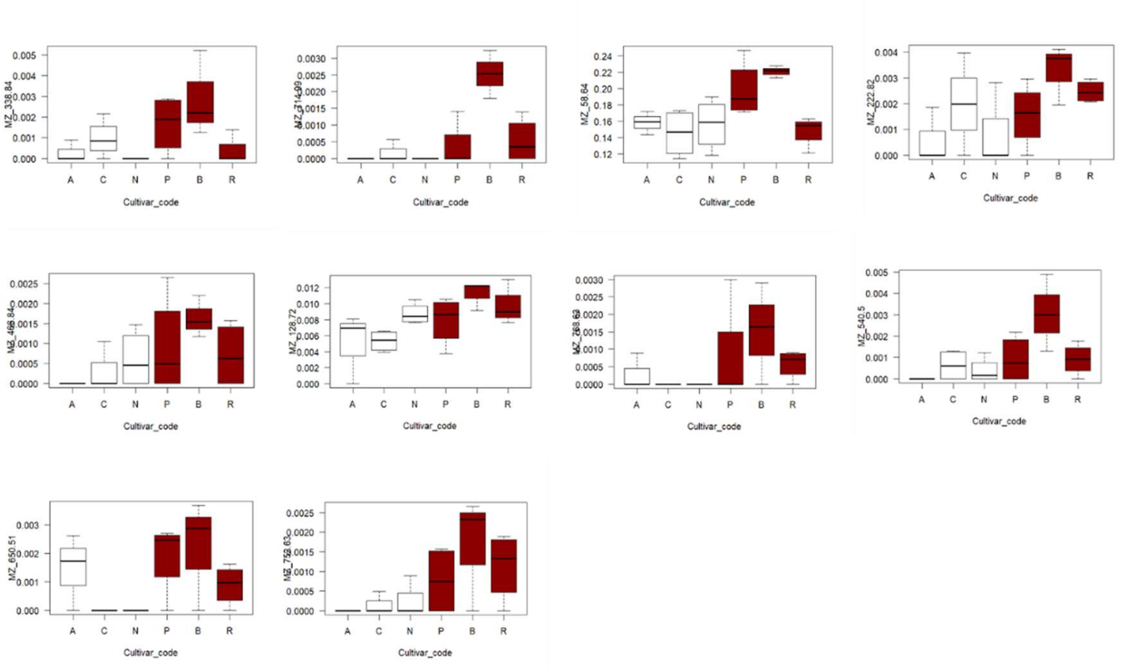
Opuntia cultivars have previously shown different flavonoid profiles (Santos-Zea et al., 2011) as well as polyphenols (Guevara-Figueroa et al., 2010). *O. robusta* is a wild species that may have more compounds related to plant defense and the 'Rojo Pelón' (domesticated cultivar) may have genetic linkages with *O. robusta*. Reyes-Aguaero et al. (2005) suggested that the systematic relation of *O. ficus-indica* with other species needs to be studied. Some anti-insect metabolites are reported by León-Solano et al., (2021).

3.2 Metabolic variations of Opuntia cultivars at after infestation

Cultivars showed differences in metabolite composition after infestation and about 37 compounds found with significantly higher abundance (Figure 3 a and b) and seven metabolites were with lower abundance (Supplemental Table 2).



(a)



(b)

Figure 3. Relative abundance of selected insect resistance discriminant features (VIP≥1.5) present in the metabolic profile of infested *Opuntia* cultivars identified with PLS-DA. The box plots present summarized data of susceptible (white) and resistant (red) cultivars. A, Atlixco; B, Bioplástico; C, Chicomostoc; N, Nopalea: P, Rojo Pelón; R, Robusta.

A large number of secondary metabolites may be appeared after infestation either because of mechanical damage or the insect (Harborne, 2007; Sandhyarani and Rani, 2013; Bennett et al., 1994) or mechanical damage (Huang et al., 2013; Tiago et al., 2017; Harborne, 2007). There can be diverse secondary metabolites that defend synergistically (Bode et al., 2013). About 33 metabolites are detected to appear in more quantity after infestation conditions within the resistant cultivars. Some of them are: mz_88.67, mz_249.82, mz_600.79, mz_768.63, in all resistant cultivars, and mz_128.7, mz_466.84, mz_540.5, mz_650.5, mz_700.6, in cv. 'Bioplástico' (Figure 3a and b).

3.3 Differences between matured cladodes and young cladodes of susceptible cultivars

PLS-DA analysis showed 37 discriminant metabolites between matured cladode at before infestation and young cladodes' metabolic profile (Supplemental Table 3). Seven metabolites (mz_61.59, mz_79.57, mz_581.02, mz_590.65, mz_592.68, mz_660.5, and mz_664.69) expressed higher in young plants of all susceptible cultivars (Figure 4). Differences in some secondary metabolites were reported by (Figueroa-Perez et al., 2018; López-Palacios and Pena-Valdivia, 2020; Hernández-Becerra et al., 2022). The contents can also be affected by the genetics and environment (Navarrete et al., 2021). The authors also noted similarities in some flavonoids. More 4-hydroxybenzoic acid and chlorogenic acid in *O. robusta* (León-

Solano et al., 2021) and more hydroxycinnamic acid in *O. ficus-indica* were reported (López-Palacios and Pena-Valdivia, 2020).

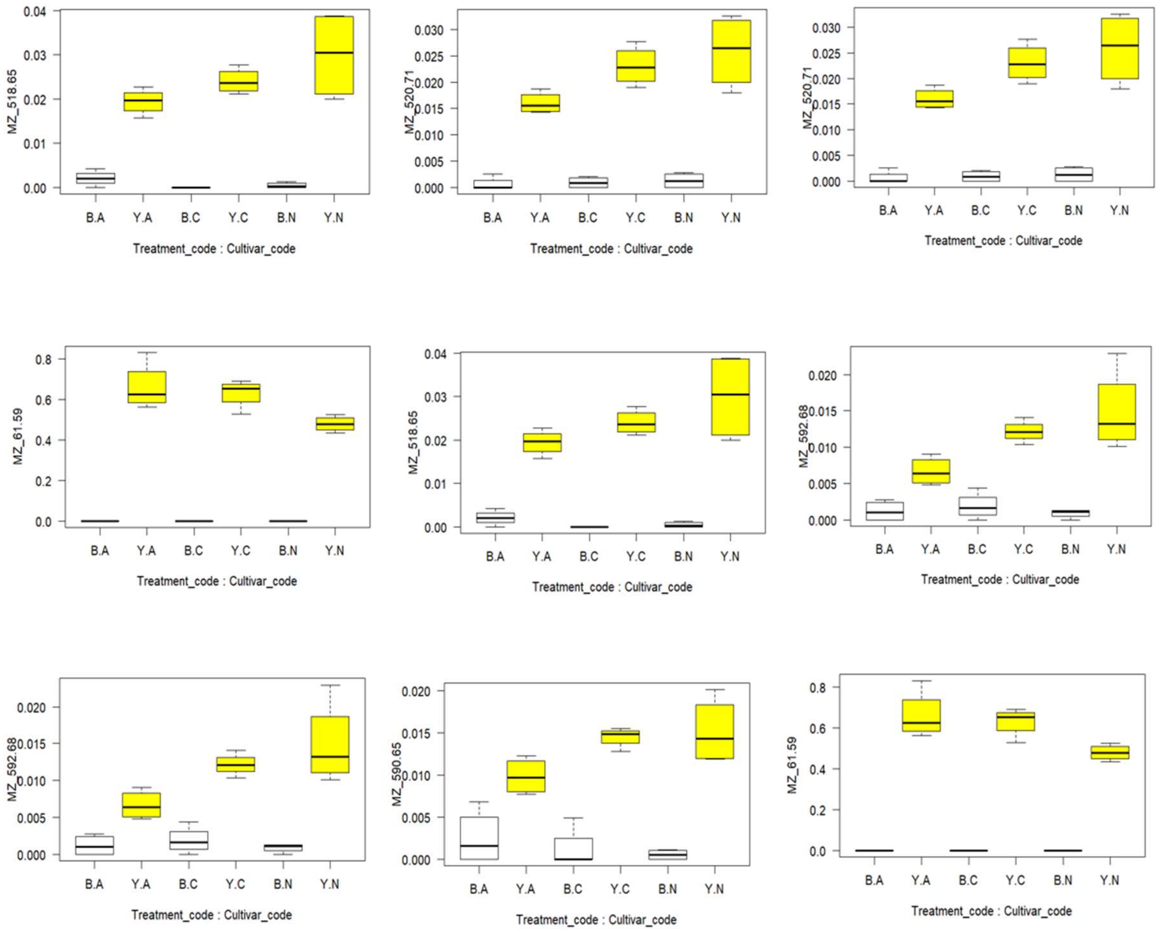


Figure 4. Relative abundance of selected insect resistance discriminant features (VIP≥1.5) present in metabolic profile of insect susceptible *Opuntia* cultivars in young cladodes identified with PLS-DA. The box plots present summarized data of matured cladode (white) and young cladode (yellow) cultivars. A, Atlixco; B, Biolástico; C, Chicomostoc; N, Nopalea: P, Rojo Pelón; R, Robusta.

PLS-DA analysis showed 37 discriminant metabolites between matured cladode before infestation and young cladodes' metabolic profile (Supplemental Table 3). Nine metabolites were expressed higher in young plants (Figure 4).

4 Discussion

Plants produce various organic compounds that appear to have no direct role in growth and development. These compounds or chemicals directly harm herbivore performance through one or two devices: they may decrease the nutritive value of the plant or they may act as feeding preventions or poisons (War et al., 2015).

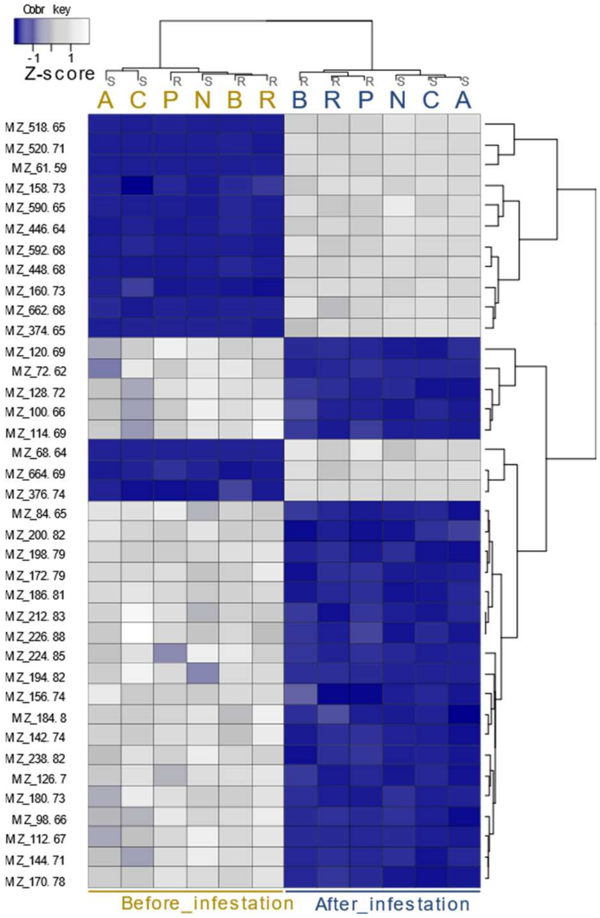


Figure 5. Hierarchical clustering showing a heat map of metabolites (VIP value ≥1.5) of non-infected and infested. Colors represent the concentration of the compounds, white-more concentration and blue-less concentration. Columns are presenting samples and rows are exhibiting metabolites. Clustering is observed from the shown dendrograms.

The use of DELI-ESI MSprofiling revealed that there is variation between the resistant and susceptible groups. Interestingly, 'Rojo Pelón' and 'Robusta', which have similar insect reactions in resistance are clustered together in infested conditions (Figure 5). This gives a clue that they have common and resistance-related metabolites. Some insect defense-related secondary metabolites are studied in *O. robusta* in previous research (Janczur et al., 2021). Differences in secondary metabolites between resistant and susceptible cultivars of cactus pear can be evidence of the fact that the plant came through a long evolutionary process and has a chemical defense system. The feeding of insects in plants, according to coevolution theory, is a determining factor in increasing the diversity of species in the hosts. And changes in the host cause changes in herbivory. If the evolution of one species results in the evolution of another, it is very likely that they are involved in a coevolutionary process known as the arms race (Fürstenberg-Hägg et al., 2013). Among Cactaceae, *Opuntia* has high diversity which is an extensively disseminated genus in the Americas that came through evolutionary success (Reyes-Aguero et al., 2005; Ernst et al., 2019). It was also observed that some metabolites are differently higher in 'Bioplástico' cultivar (Figure 3b and Figure 4b) which has different reactions to insect herbivory (Berhe et al., 2022). The authors explained that insects remained at nymph stage without molting, while other resistant cultivars died in a few weeks.

Mendez-Gallegas, et al. (2010) suggested that *Opuntia* cultivars may have defense strategy to *D. coccus*.The authors noted that this behavior may be linked to chemicals (presence of secondary compounds). Matos et al. (2021) reported that some secondary metabolites, which may be strongly associated with resistant forage palm species. The authors also noted that among the studied species, *O. cochenillifera* and *O. stricta* are resistant to wild cochineal (*D. opuntia*e). In agreement with this, among the three cultivars of *O. ficus-indica* tested, the 'Rojo Pelón' is resistant to the cochineal; and 'Bioplástico' cultivar is resistant to *D. coccus* from the *O. cochenillifera* species (Berhe et al., 2022).

A report by Wang et al. (2021) made on a study on bulbs of *Lilium davidii,* indicated that species may have similar profile of secondary metabolites regardless of environmental changes. Similarly, differences in some secondary metabolites are also reported in the white and purple sesame flowers (Dossou et al., 2021). Metabolic changes were identified in three different *Physalis* species (Trujillo-Pahua et al., 2021), secondary metabolite biosynthetic processes in *Tetranychus cinnabarinus* resistant cassava (*Manihot esculenta* Crantz) varieties (Yang et al., 2020) and difference of rice cultivars in metabolites in response to brown planthopper were reported, and rice plants respond to BPH attack by releasing various endogenous metabolites like proteinase inhibitors, callose, secondary metabolites (terpenes, alkaloids, flavonoid, etc.) and volatile compounds (Muduli et al., 2021; Roland et al., 2017). The above findings support our findings of this experiment that we noticed differences in secondary metabolite content between resistant and susceptible cactus cultivars. In addition, the presence of unique metabolites in 'Bioplástico' cultivar indicates that it may have molt deterring secondary metabolites (War et al., 2020).

In conclusion, metabolic changes between *D. coccus* resistant and susceptible groups of cactus cultivars were identified with DLI-ESI MS. Each cultivar also displayed a species-specific metabolite especially related to specific herbivory response. The findings of this study also illuminated the metabolite variation present in different cactus cultivars. This trait will require further efforts to identify highly marker metabolites that could be used as efficient tools for plant breeding programs designed to develop insect (*D. coccus*) resistance in *O. ficus-indica*. There is thus a need to understand herbivore-specific signal molecules, their mode of action, and signal transduction, as understanding plant/insect interactions is crucial for pest management (War, et al., 2012). In addition, metabolic profiles varied at non-infested and infested, which may be due to the presence of inductive and intrinsic resistance. The young cladodes showed different metabolites from the matured cladodes, which might be related to insect resistance.

5 Conflict of Interest

Authors have no conflict of interest.

6 Author Contributions

YKB, AB, IDMC designed experiments. YKP, AB, CVH perform the experiment. RW, HAP, LS did the chemical analysis and data treatment. YKB, VT, IDM conducted data analysis. YKB wrote the manuscript draft. YKB, AB, and IDM did the review and editing. All authors commented on the manuscript and agreed the submitted version.

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Supplemental Tables

Supplemental Table 1. Metabolites discriminating the metabolic profile of *Dactylopius coccus* resistnt and susceptible *Opuntia* cultivars at non-infested condition

No	Metabolite	X	No	Metabolite	x	No	Metabolite	x
1	MZ_80.63	1.592385	14	MZ_263.92	1.775364	27	MZ_576.68	1.471793
2	MZ_82.66	1.816758	15	MZ_292.87	1.664595	28	MZ_610.92	1.76731
3	MZ_92.65	2.049464	16	MZ_322.82	1.741251	29	MZ_612.62	1.572715
4	MZ_94.63	1.750087	17	MZ_326.92	2.448121	30	MZ_638.41	1.841723
5	MZ_98.66	2.347435	18	MZ_348.78	1.553452	31	MZ_650.51	1.47822
6	MZ_114.69	1.466671	19	MZ_399.2	1.94887	32	MZ_672.47	1.777884
7	MZ_128.72	1.493777	20	MZ_420.91	1.614383	33	MZ_744.79	1.648725
8	MZ_134.68	2.156994	21	MZ_437.91	1.710207	34	MZ_758.63	2.291078
9	MZ_150.72	1.907206	22	MZ_479.06	1.489037	35	MZ_776.48	1.488335
10	MZ_152.73	1.616733	23	MZ_496.84	1.789685	36	MZ_812.69	1.749282
11	MZ_158.73	2.077504	24	MZ_512.7	1.556782	37	MZ_859.32	1.599798
12	MZ_254.93	1.968762	25	MZ_538.78	1.63175	38	MZ_866.99	1.614328
13	MZ_256.9	2.554791	26	MZ_556.57	2.307901			

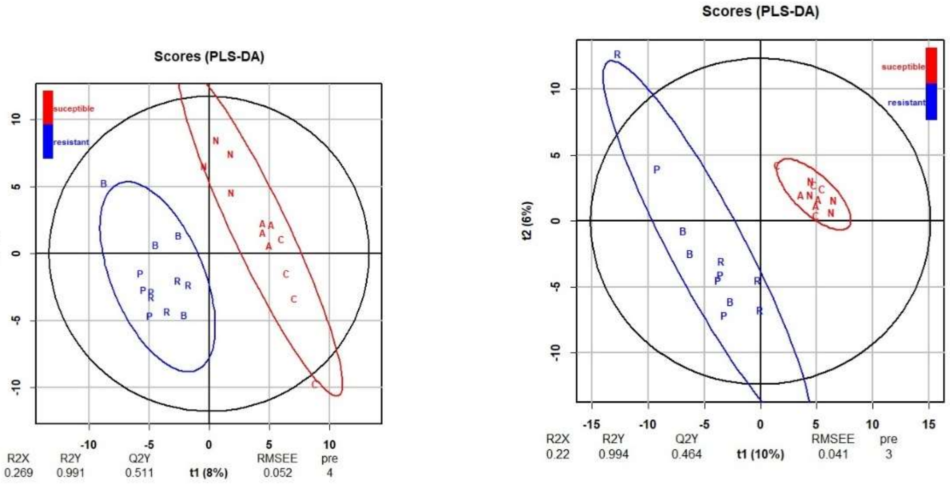
Supplemental Table 2 Metabolites discriminating the metabolic profile of *Dactylopius coccus* resistnt and susceptible *Opuntia* cultivars when infested with the insect

No	**Metabolite**	**x**	**No**	**Metabolite**	**x**	**No**	**Metabolite**	**X**
1	MZ_58.64		13	MZ_270.95		25	MZ_558.75	
2	MZ_88.67		14	MZ_282.96		26	MZ_566.89	
3	MZ_92.65		15	MZ_316.84		27	MZ_572.64	
4	MZ_114.69		16	MZ_338.84		28	MZ_600.79	
5	MZ_118.67		17	MZ_384.83		29	MZ_648.69	
6	MZ_128.72		18	MZ_394.9		30	MZ_650.51	
7	MZ_132.71		19	MZ_436.42		31	MZ_700.67	
8	MZ_134.68		20	MZ_446.64		32	MZ_714.99	
9	MZ_178.74		21	MZ_464.75		33	MZ_720.59	
10	MZ_200.82		22	MZ_466.84	1.848018	34	MZ_752.63	
11	MZ_222.82		23	MZ_518.65	1.829689	35	MZ_768.63	
12	MZ_249.93		24	MZ_540.5	1.770084	36	MZ_662.68	
						37	MZ_664.69	

Supplemental Table 3. Metabolites discriminating the metabolic profile of young and matured cladodes of *Dactylopius coccus* susceptible Opuntia cultivars

No	**Metabolite**		**No**	**Metabolite**		**No**	**Metabolite**
1	MZ_61.59		13	MZ_114.69		25	MZ_502.6
2	MZ_79.57		14	MZ_116.68		26	MZ_518.65
3	MZ_86.65		15	MZ_118.67		27	MZ_520.71
4	MZ_88.67		16	MZ_120.69		28	MZ_581.02
5	MZ_92.65		17	MZ_122.69		29	MZ_590.65
6	MZ_94.63		18	MZ_126.7		30	MZ_592.68
7	MZ_96.66		19	MZ_128.72		31	MZ_660.55
8	MZ_100.66		20	MZ_144.71		32	MZ_664.69
9	MZ_102.65		21	MZ_230.72		33	MZ_700.67
10	MZ_104.67		22	MZ_250.8		34	MZ_716.61
11	MZ_110.69		23	MZ_254.93		35	MZ_720.59
12	MZ_112.67		24	MZ_280.77		36	MZ_810.68
						37	MZ_866.99

Supplemental Figures



(a) (b)

Supplemental Figure 1. PLS-DA score plots of cladode metabolites profile in resistant (blue) and susceptible (red) cultivars *Opuntia* cultivars at before infestation (a) and after infestation (b). A, Atlixco; B, Bioplástico; C, Chicomostoc; N, Nopalea: P, Rojo Pelón; R, Robusta.

4.3 Análisis UFLC de metabolitos secundarios de cultivares de *Opuntia ficus-indica* resistentes a *Dactylopius coccus* Costa

UFLC analysis of secondary metabolites of *Opuntia ficus-indica* cultivars resistant to *Dactylopius coccus* Costa

UFLC based analysis of secondary metabolites of *Opuntia ficus-indica* cultivars resistant to *Dactylopius coccus* Costa

Abstract

Dactylopius coccus is a very important pest of *Opuntia ficus-indica* in different parts of the world. The management technique is recommended to be an integrated approach that comprises the use of resistant cultivars. The mechanism of resistance can be different, and among them is having defense secondary metabolites. Metabolomic analysis was done to assess if some defense-related plant secondary metabolites are present in *D. coccus*-resistant *O. ficus-indica* cultivars, with ultrafast liquid chromatography (UFLC). Samples from six cultivars at different herbivore damage conditions were collected in four replications. Results indicated that quercetin and apigenin appeared in significant quantity in Robusta and Bioplástico cultivars respectively. These both cultivars are resistant to *D. coccus*.

Introduction

Dactylopius spp (Hemiptera: Dactylopiidae) are grave pest of cactus pear, *Opuntia ficus-indica* (Mazzeo et al., 2019; Foldi 2001; Spodek et al., 2014; Ben-Dov and Sánchez-Garcia, 2015; Bouharroud et al.,2016; Berhe et al., 2020; Fitiwy et al., 2016). *D. coccus* damaged significant areas of plantations in Tigray (Belay 2015, Berhe et al., 2020), and has a significant effect on cactus pear cultivation (MacGregor & Sampedro,1984).

D. opuntiae occurs, but mechanisms of resistance are not addressed yet. In Brazil, in laboratory and field tests, some varieties have shown resistance to the *D. opuntie* (Passos da Silva et al., 2009; Vasconcelos et al., 2009; Borges et al.,2013).

Secondary metabolites help plants in insect defense (War et al. 2012). *O. ficus-indica* is rich in natural bioactive chemicals such as carbohydrates, flavonoids, mineral amino acids (arginine), phenolic acids (caffeic and gallic), polyphenols, quercetin, tannins, tocopherol, and sulfur amino acids (cysteine, methionine, and taurine) (El-Hawary et al., 2020; Avila-Nava .2014; Pooja et al, 2006; Saleem et al., 2006; Nassrallah et al., 2021). Some secondary metabolites found can be defensive against *D. coccus* (Lopez-Palacio and Pena-Valdiva, 2020). Differences among genotypes are noticed by different authors (Farag et al., 2020; Lopez-Palacio and Pena-Valdiva, 2020; Francisco et al., 2016).

Total polyphenols and total flavonoids increase the antioxidant capacities of cactus (Silva-Beltrán et al., 2015). Role of flavonoids to protect plants from different biotic and abiotic stresses and function as signal molecules, allopathic compounds, phytoalexins, detoxifying agents, and antimicrobial defensive compounds is known (Panche et al., 2016). Flavonoids affect insect herbivory which depends on the concentration the plant has in it (Samanta et al., 2011). In soybean *Glycine max* (L.) Merr. (Fabaceae) cause various modifications in the metabolism, behavior, and development of insect herbivores (Stec et al., 2021). Lu et al. (2017) reported that the total flavonoid content reduced the expansion rate of the disease spot area in rust-resistant Malus plants.

O. ficus-indica, plants are known for its high-level polyphenols and flavonoids (Silva-Beltrán et al., 2015). Flavonoids affect insect herbivory, which depends on the plant's concentration (Samanta et al., 2011). Lu et al., (2017) reported that the total flavonoid content reduced the increase rate of the disease spot area in rust-resistant Malus plants. Winter triticale (Triticosecale *Wittm*) plants induce secondary metabolites when they are damaged by grain aphid (Sitobion avenae F.) (Chrzanowski and Leszczyński, 2008,). In resistant sorghum (*Sorghum bicolor* (L.), flavonoid (3-deoxyanthocynadin) inflicted significantly higher mortality and reduced population growth in corn leaf aphid (*Rhopalosiphum maidis* Fitch) (Kariyat et al., 2019). The growth and fitness of adult tobacco hornworm, *Manduca sexta* L negatively impacted polyphenol-rich maize varieties (Tayal et al., 2020). Polyphenols are important defensive chemicals to many insects (Tayal et al., 2020).

Therefore, this study is aimed at investigating the phytochemical difference specifically; the content of hydrogen peroxide, total protein, total polyphenols, and flavonoids.

Materials and Methods

Plant material and sample preparation

Six *Opuntia* cultivars consisted of three *O. ficus-indica* (‘Rojo Pelón’, ‘Atlixco’, and ‘Chicomostoc’), two *O. cochenillifera* (‘Nopalea’ and ‘Bioplástico’) and one *O. robusta* (‘Robusta) was studied (Table 1). The field-collected cladodes were thoroughly washed with clean tap water. The matured, vigorous, and diseases and insect-damage-free cladodes from each cultivar were used for the experiment. Experimental design was completely randomized design (CRD) and done in a greenhouse. There were two treatments (Control (undamaged) and Herbivore Damaged (HD)), each treatment consisted of four biological replicates, totalling eight cladodes per cultivar, and the four replicates were used as a control, and the remaining four used for HD. The control and HD cladodes were numbered and marked with their respective treatment, replicate, and cultivar.

Insect Infestation

Adult *D. coccus* females reared in separate cladodes were prepared for the HD cladodes. First, the 20 adult females were removed from rearing host cladodes with a fine brush and kept in small paper bags. Next, the paper bags containing 20 adult females each were hooked with spines of their respective replicates and cultivars. Next, infested cladodes were covered with nylon clothing bags to protect them from uncontrolled infestation and pests. Mata and Carlos (2013) described that the paper bags remained attached to the cladodes for seven days and were then removed. Then, after approximately 100 days, the insect's life cycle is completed on the susceptible cultivars, and samples from the control and HD cladodes were collected into liquid nitrogen using a punching instrument and after that stored at -80°C. Fresh-frozen plant tissues were then lyophilized and homogenized. Finally, finely powdered plant tissue was weighed in polypropylene microfuge tubes and used for the following analysis.

UFLC profiling of secondary metabolites

The analysis was carried out with a UFLC system (LC 20AD, Shimadzu Corporation, Japan) equipped with a photodiode array (PDA) detector (SPD, M20A, Shimadzu Corporation, Japan). Compounds separation was done using a reversed-phase C18 column (4.6 x 250 mm, 100 A, 5µm particle size, Phenomenex, India). A gradient binary mobile phase consisting of 0.1 % phosphoric acid (v/v) as solvent A and acetonitrile in solvent B at a flow rate of 30 µL/min was used with an initial A: B ratio of 90:10 for 25 min, in an equilibration step with initial concentration for 5 min for a total run time of 25 minutes detection and tentative identification of specific phenolic acid analysis was carried out using the diode array data between 210 to 700 nm along with their retention times and the concentrations were expressed in terms of mg/gm of flour (Omagamre et al., 2022).

Results and Discussion

UFLC profiling of some secondary metabolites

From the suspected secondary metabolites, quercitin in ‘Robusta’ cultivar and apigenin in ‘Bioplastico’ cultivar were detected with higher intensity at before HD. Valine was higher in Robusta after HD (Figure 1). Some other compounds are detected in many samples (Table 1).

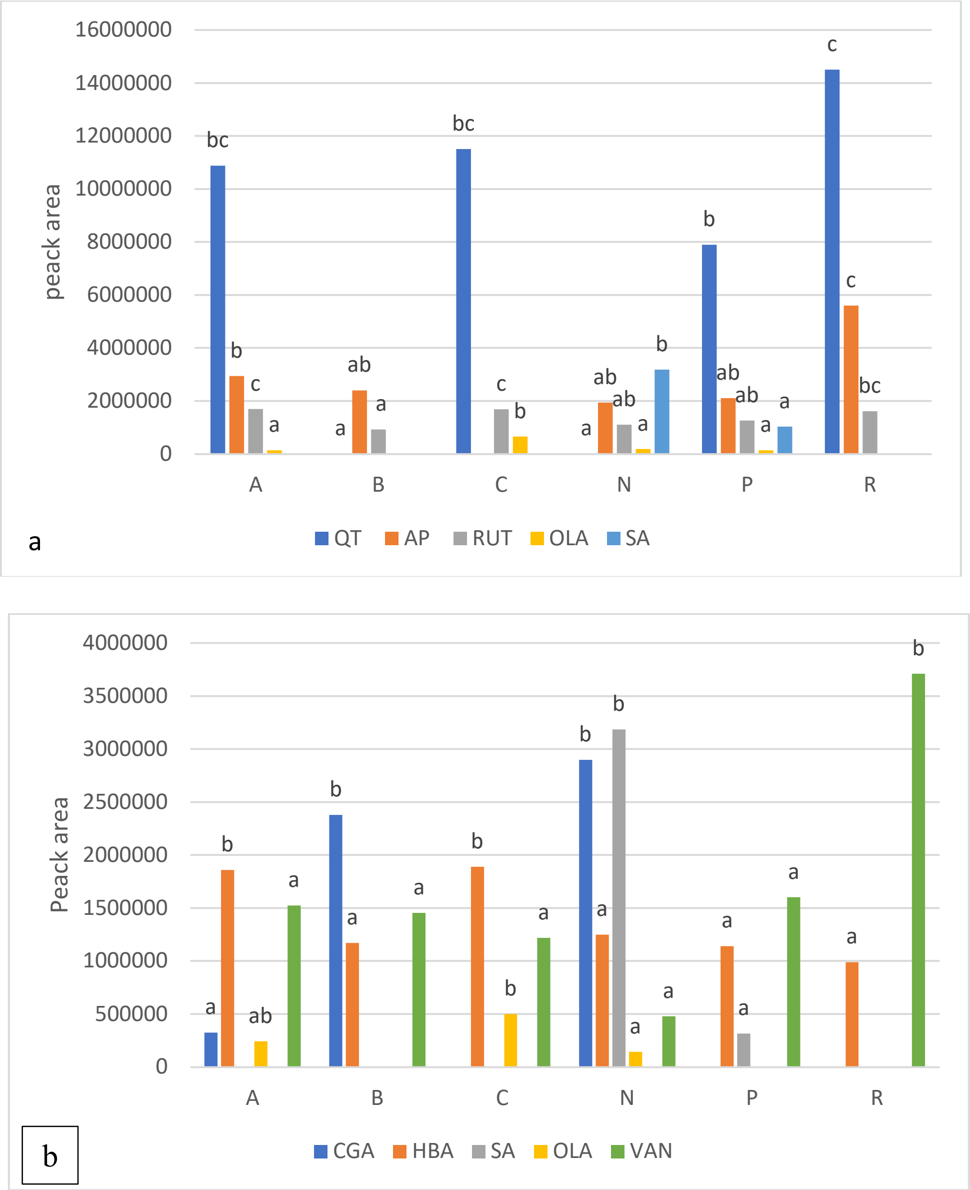


Figure 1. Some secondary metabolites were profiled from six *Opuntia* cultivars and their peak areas. a, before herbivore damage, and b, after herbivore damage. A-Atlixco; B-Bioplastico; C-Chicomostoc; N-Nopalea; P-Rojo Pelon; R-Robusta; QT-quieroctine; AP-Apiginine; RUT-rutine; OLA-oleonelic acid; and SA-Syringic acid.

Table 1. Other compounds detected with no significant variation among cultivars

	Before herbivore damage					After herbivore damage					
CV	CGA	FA	VA	HBA	VAN	CFA	FA	VAN	QT	RUT	AP
A	x	-	-	x	-	X	-	X	x	x	x
B	x	x	-	x	-	X	x	X	-	-	x
C	-	-	x	x	-	X	-	X	x	x	x
N	x	-	x	x	-	X	-		-	x	x
P	x	-	x	x	-	-	-	X	x	-	x
R	x	x	x	x	-	X	x	X	x	-	x

A-Atlixco; B-Bioplastico; C-Chicomostoc; N-Nopalea; P-Rojo Pelon; R-Robusta

‘Robusta’ cultivar showed significant differences in quercitin and apigenin before HD and vanillin after HD. However, the other two cultivars didn’t show superior in all suspected secondary metabolites (Figure 6). Qualitative analysis also shows ferulic acid was detected only at two resistant cultivars (‘Bioplastico’ and Robusta’) at both HD conditions (Table 4).

In line with these findings, Matos et al., (2021). reported that some secondary metabolites like quercetin3-O-2’,6’-rhamnosyl glucoside, quercetin rhamnosyl dihexoside, not identified, isorhamnetin-glucoside i-rhamnoside and isorhamnetin-3-sophoroside7-rhamnoside, which may be strongly associated with *D. opuntia* resistance in nopal species. The presence of quercetin in ‘Robusta’ was also reported by Janczur et al. (2021). Flavonoids are important plant secondary metabolites, which protect plants from herbivory damage (War, Sharma, & Sharma 2016). Different varieties of the *Opuntia* genus can have different flavonoid compounds (Santos-Zea et al., 2011).

Chlorogenic acid protects a broad range of insects by limiting growth (Kundu and Vadassery, 2019) and can be used in pest control strategies (War et al., 2013). Hydroxycinnamic acids, caffeic acid, chlorogenic acid, ferulic acid, syringic acid, vanillic acid has insect defense role in different plants (Lopez-Palacio and Peña-Valdivia, 2020; Rocchetti et al., 2018). Apigenin also has both anti-herbivore and antimicrobial effects in plants (Golawska et al., 2010; Jiang et al., 2012; Bilska et al., 2018). Antimicrobial plant compounds like polyphenols indirectly disturbs the insect herbivory by disturbing beneficial microbes associated with insects (Singh et al., 2021). Cochineals have symbiotic microorganisms which help to produce amino acids (Vera-Ponce de Leon et al., 2017; Rosenblueth et al., 2018; Vera-Ponce de Leon et al., 2016; Ramírez-Puebla et al., 2016). Ferreira et al. (2021) concluded that *O. ficus-indica* cladodes contain flavonoids that are toxic to eggs and larvae of *Plutella zylostella* and can obstruct oviposition (Ferreira et al., 2021).

Conclusions

Many secondary metabolites which have a defense role in other plants were detected in this experiment. However, only quercetin and apigenin were detected in resistant cultivars with significantly higher amounts.

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CAPÍTULO 5. MICROPROPAGACION Y CALIDAD DEL FORAJE DEL CULTIVARES DE *Opuntia ficus-indica* RESISTANTES A *Dactylopius coccus* COSTA

5.1 Propagación *in vitro* de *Opuntia ficus-indica* cv. Rojo Pelón resistente a *Dactylopius coccus* Costa

Berhe Y.K, Portillo L. and Soltero R. 2023. *In vitro* propagation of resistant *Opuntia ficus-indica* cv. ‘Rojo Pelón to *Dactylopius coccus* Costa. Vegetos

Somatido: 14 de mayo de 2023

Vegetos

In vitro propagation of Dactylopius coccus Costa resistant Opuntia ficus-indica cv. Rojo Pelón

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Author Comments:	My manuscript has important results of protovol adapataion for tissue culture of Dactylopius coccus resistant cultivar of Opuntia ficus-indica.

In vitro propagation of *Dactylopius coccus* Costa resistant *Opuntia ficus-indica* cv. Rojo Pelón

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Abstract

Opuntias can be propagated *in vitro* media. However, different species and cultivars need a specific protocol for efficient propagation. Therefore, *in vitro* protocol adaptation study was done on the *Dactylopus coccus* Costa resistant, 'Rojo Pelón' cultivar with different trials. The proliferation of new shoots was low with BA hormone, while other cultivars produced more shoots. However, MS media with kinetin growth regulator induced shoot proliferation, and the best shoots in number and quality were observed in 7 mg/L of kinetin. The average number of shoots produced was 4.5. *Ex vitro* test resulted in a 66.6 to 100% establishment rate of the plantlets. The cultivar can be propagated with the addition of 7 mg/L of kinetin to MS media. The need for cultivar-based protocol adaptation and optimization was proven in this study.

Keywords: Proliferation; micropropagation; shoot; hormones, protocol

Introduction

Opuntia plants adapt to marginal environments and resist different abiotic stress (Blanco et al. 2003). Plant tissue culture is an important technology to produce/multiply plants massively and 'true to type' clones with the desired characteristic (Karp 1995; Kulus 2014). It is also a beneficial tool for maintaining endangered species. *Ex situ* conservation is an essential strategy to conserve the Cactaceae family (Hubstenberger et al. 1992; Santos-Díaz et al. 2010), and *in vitro* culture is also important to preserve plant materials for years (Zoghlami et al. 2012; Bhowjwani and Dantu 2013). Such type of conservation system reduces the overutilization of threatened plant species (Pérez-Molphe-Balch et al. 2012). Another importance of *in vitro* culture is for secondary metabolite production (Robel-Martinez et al. 2016; Espinosa-Leal, 2018).

Successful *in vitro* propagation for different cacti has been developed (Fay et al. 1992); for several cactus pear genotypes used as vegetable sources for human consumption effective micropropagation protocols have been developed (Mohamed-Yasseen et al. 1995; Pedro, *et al.* 2005). *Opuntia ficus-indica* can be effectively micro-propagated by areoles, and easily acclimatized to field conditions as the best strategic means to ease desertification in dry regions (Khalafalla 2007; Bouzroud and Sobeh 2022).

However, diverse factors such as genetics and growth regulators influence them (Bouzroud and Sobeh 2022).

Benzyladenine (BA) growth regulator helps with the shoot production of regenerated cactus pear (Angulo-Bejarano and Paredes-Lopez, 2011). Best multiplication rates of *O. ficus/indica* cultivars were recorded with the addition of BA (El Finti et al. 2013; de Medeiros et al., 2006), medium and best results were observed in Murashige and Skoog (MS) media supplemented with 5 mg/L BA (Khalafalla et al. 2007; El Finti 2013). Gracia-Saucedo et al. (2005) also reported 0.5 µM of BA gave the best proliferation of cactus. Ghaffari et al. (2013) reported that the best shoot development and elongation of cactus pear were obtained in media containing 5 mg/L 6-Benzylaminopurine (BAP).

Different species and genera respond in different ways to growth regulator types and concentrations. A combination of BA and Naphthalene acetic acid (NAA) can give more shoots proliferation in *Cereus peruvianus* (Cactaceae) (Machado and Prioli 1996), *Opuntia milpa-alta* (Lijing et al. 2001), and *Sulcorebutia alba* (Debekaussen et al. 1991). In another way, *in vitro* cultured *Coryphantha elephantidens* cactus proliferated with BAP supplemented media (Bhau and Wakhlu 2015). *O. fragilis* was best proliferated at a mixture of BA and Indole-3-butyric acid (IBA) (Vidican and Cachita-Cosma 2010). BAP and IBA supplemented media were used for *in vitro* culture of *O. ellisiana* (Juarez and Passera 2002).

Differences among cultivars were observed in response to the different stimulants or growth regulators (Garcia-Saucedo, et al. 2005). Differences in shoot number, shoot length, root number, and root length were recorded among six genotypes in Tigray (Mengesha et al. 2016), and among three genotypes in Morocco (El Finti et al. 2013a), The variation in cultivars response to media conditions, indicates that importance of optimizing the growing conditions to maximize the value of different cultivars (Bougdaoua and El Mtili 2020).

Evaluation and multiplication of selected varieties/cultivars in research stations and tissue culture laboratories are the key activities that need to be conducted to boost the sustainable development of *Opuntias* (Ozturk et al. 2019; Pathak and Abido 2014). 'Rojo Pelón' cultivar is cochineal resistant (Berhe et al. 2022) and needs to be adapted with *in vitro* micropropagation protocols. Different protocols for different species and clones of cacti must use specific micropropagation protocols (Lema-Ruminska and Kulus 2014; Soltero and Portillo 2015). Thus, this experiment was conducted to evaluate the micropropagation performance of this cochineal-resistant cultivar in different growth regulators and rates.

Material and Methods

In vitro culture

Tissue culture protocol for *Opuntia* cultivars was performed according to (Gutiérrez-Quintana et al. 2018).

Establishment of the *in vitro* culture

Cultivars of *O. ficus-indica* (‘Atlixco’, ‘Teponahusco’, and ‘Rojo Pelón’) were collected from the field and kept in a greenhouse for new shoot development. Young cladodes about 3 to 4 weeks of age from sprouting, were taken from a greenhouse plantation and were cut into pieces containing at least five areoles for proliferation. And the pieces were surface sterilized by washing under running tap water, then immersed in 70% ethanol for 10 seconds, washed in 30 mL bleach and 30 ml water solution for 10 min, and then immersed in water three times. Then the inoculation was carried out at an MS media containing *in vitro* glass (Figure 1), and it was placed in a growth chamber containing a 16 h light/8 h darkness photoperiod at a light intensity of 25 µmol photons/m^2/sec, at 25 °C.

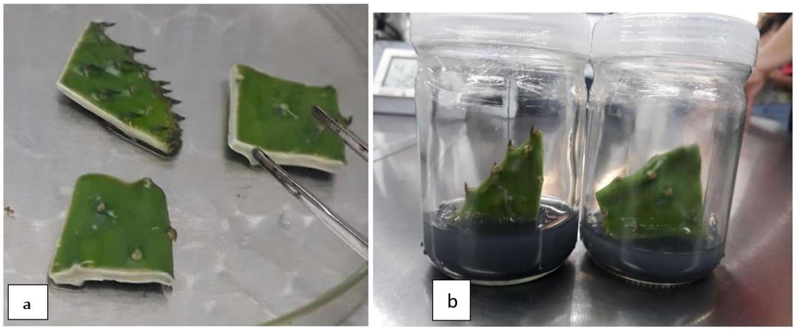

Figure 1. Sterilized fraction of young cladodes (a) and *in vitro* inoculated shoots with MS media containing 4 g/L of activated charcoal (b)

The proliferation of plant material

Then explants were transferred to a container with a stimulation medium with the apical meristem facing up. The growth regulator in the medium stimulated axillary shoot proliferation from the basal meristems. During experiment one, four cultivars were tested under 2.2 mg/L BA. Then, experiment two was continued with kinetin (0, 5, 10, and 15 mg/L). The final experiment was done with kinetin levels (5, 6,7,8,9, and 10) mg/L. All experiments were conducted in four replication and a completely randomized design. The collected data were subjected to analysis of variance (ANOVA), and mean comparisons when differences among treatments were observed.

Ex vitro establishment

The *in vitro* plantlets, which were proliferated with different kinetin hormones, were monitored for rooting and then prepared for *ex vitro* conditions. Small spines developed, and the surface acquired a dark-green color. Then it was transferred to a nursery tray with a proper substrate. The survival rate was counted, and the average value of each treatment was calculated.

Results and Discussion

Testing proliferation with BA supplemented media

Cultivars showed significant differences in the number of proliferated shoots (p=0.0000). ‘Atlixco’ scored the highest shoot number and ‘Rojo Pelón’ is the lowest (Figure 2). The differences among genotypes or cultivars proliferation under a certain media conditions are supported by previous studies.

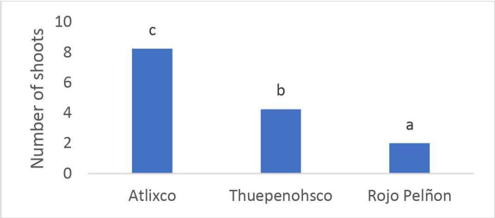

Figure 2. Number of proliferated shoots of three *Opuntia ficus-indica* cultivars under benzyl adenine (BA) growth regulator supplemented MS media.

Previous research reports agreed with the current finding, that the cultivars have a different response to the addition of growth regulators. *Opuntia* proliferation was enhanced with BA supplemented media (Estrada-Luna et al. 2008) and the proliferation of different three cultivars of *O. ficus-indica* was different in Morocco (El Finti et al. 2013a). *In vitro* performance of two varieties of *O. ficus-indica* showed variation (Bougdaoua and El Mitili, 2020) and may vary in media conditions (Karmin et al. 2010). Plant growth conditions for best proliferation vary among cacti genera and species (Balch et al., 1998). Kinetin hormone types affect the shoot proliferation of pear cultivars (Kadota and Niimi 2003).

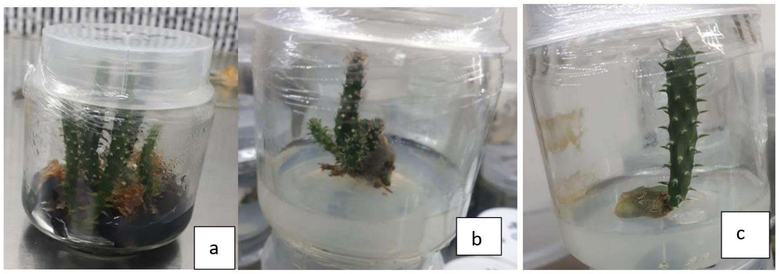

Figure 3. Proliferated shoots of three *Opuntia ficus-indica* cultivars under benzyl adenine (BA) growth regulator, a (Atlixco), b (Thuepenohsco), and c (Rojo Pelon).

Testing with Kinetin supplemented media

As demonstrated at figure 4, the treatments showed significant differences in the number of shoots (p=0.0181) but not in shoot length and vigor. T2 (5 mg/L) and T3 (10 mg/L) showed a higher number of shoots while T1 (0) showed the lowest number of shoots.

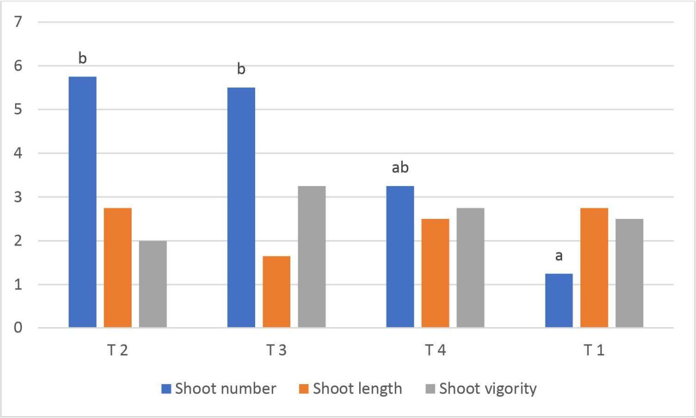

Figure 4. Number of proliferated shoots, shoot length (cm), and shoot vigorous of *Opuntia ficus-indica* cv. Rojo Pelón under different rates of kinetin hormone. T1-0 mg/L, T2- 5mg/L, T3-10mg/L and T4-15 mg/L

The importance of kinetin hormone for shoot proliferation was elaborated on before. Addition of kinetin induced shoot proliferation in *Turbinicarpus valdezianus* (Palacios et al. 2016). Gebretsadik et al. (2013) found culturing in a medium containing 2 mg/L of kinetin gives the best result for *O. ficus-indica in vitro* culture in Tigray. In an experiment conducted on cacti species, Giusti et al. (2002) reported that better number and quality of shoots proliferation on media with BA for *Escobaria minima* and *Mammillaria pectinifera*, but with kinetin for *Pelecyphora aselliformis*. The highest proliferation of shoots was recorded with the addition of 8.8 µM BA+ 30 g/l sucrose for *Pelecyphorbia aselliformis* and 8.8 µM +50 g/l sucrose for *Pelecyphorbia storbiliformis* (Perez-Molphe-Balch and Davila-Figuera, 2002). *In vitro* performance of varieties of *O. ficus indica* varied in media conditions (Bougdaoua and El Mitili 2020).

In addition, in responding to the kinetin, the proliferation varied with rates. Kadota and Niimi (2003) observed BA concentration affected pear shoot proliferation. Pitahaya varieties responded differently to BAP rate (Bozkurt et al. 2020)

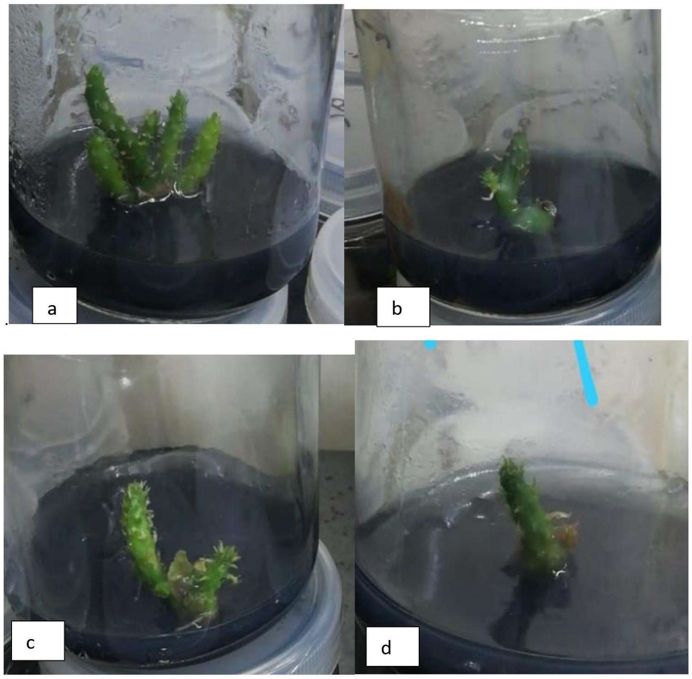

Figure 5. Proliferated shoots of *Opuntia ficus-indica* cv. Rojo Pelón under different kinetin levels, a (0), b (5 mg/L), c (10 mg/L), and d (15 mg/L).

Optimizing kinetin levels

Different kinetin levels showed significant differences in shoot vigor (p= 0.0329), but not in the number of shoots (p=0.825) and shoot length (p= 0.819). Treatments with 7 mg/L were superior in shoot vigor, though statistically similar with 8 mg/L (Figure 6). In line with this finding, Estrada-Luna et al (2008) reported higher shoot numbers in 5 and 7.5 mg/L than 1.25 mg/L of cytokinin in *O. lanigera.*

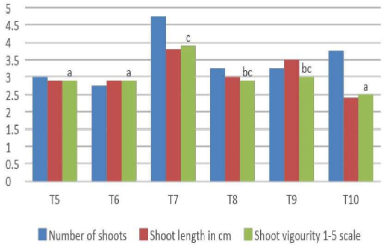

Figure 6. Number of proliferated shoots, shoot length, and shoot vigor of *Opuntia ficus-indica* cv. Rojo Pelón under different rates of kinetin hormone. T5-5 mg/L, T6 -6 mg/L, T7- 7mg/L, T8-8mg/L, T9-9mg/L, and T10-10mg/L.

Cytokine type and quantity affect the proliferation of *Prunus domestic*a (Welella and Dagnaw 2017), which affects proliferated shoot performance (length and vigor) (Ng et al. 2021; El Finti et al. 2013b). The morphological influence of kinetin on *Melocactus glaucescens* micropropagation was reported (Torres-Silva et al., 2021; Tellez-Roman et al. 2017). Bouzroud and Sobeh (2022) discussed the effects of different growth regulators on the shoot performance of cacti. Cultivars of the same species of *O. ficus-indica* can have different responses to shoot proliferation (Bougdaoua and El Mitili 2020).

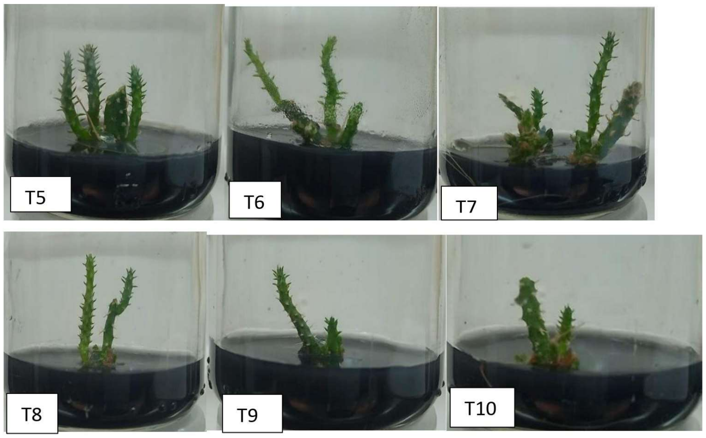

Figure 5. Proliferated shoots of *Opuntia ficus-indica* cv. Rojo Pelón under different kinetin levels, a (5 mg/L), b (6 mg/L), c (7 mg/L) and d (8 mg/L), e (9 mg/L) and f (10 mg/L).

Ex vitro establishment of plantlets

The average value of *ex vitro* establishment ranged from 60 to 100%. Healthy *in vitro* plantlets can establish in soil medium easily (Mohamed-Yaseen et al. 1995). Hasanloo and Khayam (2009) also reported 100% *ex vitro* survival of *O. ficus-indica*. However, Bouchiha and Mazri (2022) reported a survival rate of *O. ficus-indica* 81.25%

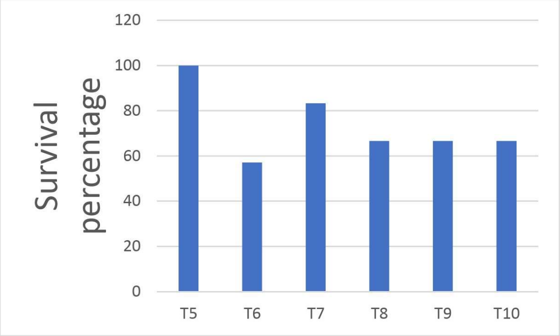

Figure 6. *Ex vitro* survival percentage of plantlets at the greenhouse of *Opuntia ficus-indica* cv. Rojo Pelón *in vitro* grown under different kinetin levels. T5-5mg/l, T6- 6mg/l, T7 -7mg/L, T8 -8mg/L, T9 -9 mg/L, and T10 -10 mg/L.

Conclusions

In vitro culture of *O. ficus indica* cultivars proliferated differently at BA supplemented media and subsequently, 'Rojo Pelón' (*D. coccus* resistant) had a low proliferation rate. However. this *D. coccus* resistant cultivar proliferated better with kinetin supplemented media. In addition, the rate of kinetin which yielded the highest shoot number with better vigor was 7 mg/l. Cultivars of *Opuntia ficus-indica* need specific protocols with the type and concentration of cytokinin for better results of micropropagation.

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Authors contributions

YKB wrote the manuscript. YKP, LP, and RS designed the experiments. Lp and RS reviewed the manuscript.

Availability of data

Not applicable

Declarations

The authors declared that they have no conflicts of interest in this manuscript.

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5.2 Nutrición y digestibilidad *in vitro* de *Opuntia ficus-indica* cv. 'Rojo Pelón' resistente a *Dactylopius coccus* Costa

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Nutrition and *in vitro* digestibility of *Opuntia ficus-indica* cv. ‘Rojo Pelón’ resistant to *Dactylopius coccus* Costa

Nutrición y digestibilidad *in vitro* de *Opuntia ficus-indica* cv. ‘Rojo Pelón’ resistente a Dactylopius coccus Costa

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Abstract

Due to its multipurpose benefits, *Opuntia ficus-indica*, or cactus pear, has been expanding from its origin, America, to different corners of the world, since the 16th century. *Dactylopius* species also spread over the last decades and created a significant loss in different countries. The utilization of resistant varieties that have desired nutritional qualities is indispensable. This study aims to evaluenutritional qualities of *D. coccus* resistant cv. ‘Rojo Pelón’. CRD design with three replications was applied and treatments were three cultivars (‘Rojo Pelón’ and two susceptible cultivars: ‘Chicomostoc’ and ‘Atlixco’). The resistant cultivar showed lower protein content than ‘Atlixco’, and higher fiber and fat content than the other cultivars, but similar in *in vitro* dry matter digestibility, compared with the susceptible cultivars. DPPH showed no significant difference among all cultivars. Flavonoid content was lower in ‘Rojo Pelón’ than ‘Atlixco’ and lower in Ascorbic acid than ‘Chicomostoc’. All the results quantified were in the range of studies made on *O. ficus-indica*. The resistant cultivar has equivalent nutritional content to the two susceptible cultivars.

Keywords: cactus pear; cultivar; nutrition; forage.

Resumen

Debido a sus múltiples beneficios, la *Opuntia ficus-indica*, o tuna, se ha ido expandiendo desde su origen, América, a diferentes rincones del mundo, desde el siglo XVI. Las especies de Dactylopius también se extendieron en las últimas décadas y crearon una pérdida significativa en diferentes países. Es indispensable la utilización de variedades resistentes que tengan las cualidades nutricionales deseadas. Este estudio se realizó para evaluar las cualidades nutricionales de D. coccus resistente cv. 'Rojo Pelón'. Se aplicó un diseño CRD con tres repeticiones y los tratamientos fueron tres cultivares ('Rojo Pelón' y dos cultivares susceptibles: 'Chicomostoc' y 'Atlixco'). El cultivar resistente mostró menor contenido de proteína que ‘Atlixco’, y mayor contenido de fibra y grasa que los otros cultivares, pero similar en digestibilidad de materia seca *in vitro*, en comparación con los cultivares susceptibles. DPPH no mostró diferencias significativas entre todos los cultivares. El contenido de flavonoides fue menor en ‘Rojo Pelón’ que en ‘Atlixco’ y menor en ácido ascórbico que en ‘Chicomostoc’. Todos los resultados cuantificados estuvieron en el rango de los estudios realizados sobre *O. ficus-indica*. El cultivar resistente tiene un contenido nutricional equivalente al de los dos cultivares susceptibles.

Palabras clave: nopal; cultivar; nutrición; forraje.

1. Introduction

Opuntia ficus-indica L. (Mill.) or cactus pear is a member of the family Cactaceae (Christenhusz & Byng 2016). It was an important crop in Mexico, during the 16th century (Griffith 2004), and the Spanish took it to their home and then expanded to the Mediterranean (Barbera et al. 1992; (Reyes-Agüero et al. 2005). It was also introduced to Tigray, in the middle 18th century (Griffith 2004; Berhe et al., 2022).

Dactylopius coccus damaged substantial areas of plantations in Tigray (Belay 2015; Berhe et al. 2020; Berhe & Siyum 2022) and *D. opuntiae* is a problematic pest in Morocco, Brazil, Israel, and other countries (Bouharroud et al. 2016; Mazzeo et al. 2019; Torres & Giorgi 2018; Spodek et al. 2014). Therefore, *Dactylopoius* species are important pests of cactus pear, and efforts are needed to combat the problem, including utilizing resistant varieties. Resistant cultivars are vital components of IPM to manage *D. coccus* in Tigray (Berhe et al. 2020) and are accessible (Teetes 1996) and manageable (Sharma & Ortiz 2002) by farmers. High productivity and pest and disease resistance are the real objectives in breeding new forage cultivars, but nutrition quality should be given emphasis (Perez-Gonzalez & Mondragón-Jacobo 2000).

Animal feed is among the important uses of cactus pear in different countries like Tigray (Ethiopia) (Brutsch 1997) and Mexico (Gallegos-Vazquez et al. 2009). Cactus pear is a good source of feed with fermentable carbohydrates and degradable protein for livestock (Vieira et al. 2008; Batista et al. 2009). The use of cactus pear as a forage for livestock in semiarid regions of Brazil was also elaborated by de Andrade Ferreira et al. (2012) & Monteiro et al. (2019). However, important nutritional quality, such as protein quality and mineral content of some cactus pear cultivars is necessary to determine (Ben Salem & Abidi 2009). Cultivars affect nutrition and ruminal degradability (Batista et al. 2009). Insect-resistant cultivars are also preferable to have competitive nutritional value (Monteiro et al. 2019), though insect resistance costs the plant because there can be morphological and physiological changes (Karban et al. 1997; Shanker et al. 2022). Mostly, resistance has yield penalties or costs (Brown 2002), due to the distribution of resources to defense and away from crop growth and development (Walters & Heil 2007). 'Rojo Pelón' cultivar possibly originated in the Central Highlands of Mexico (Cervantes-Herrera et al. 2006) and is *D. coccus* resistant. Ortiz et al. (2021) explained that cultivars 'Copena', 'Pelón Blanco', and 'Rojo Pelón' of *O. ficus-indica* comprise the largest crop surface area recorded at the Potosi highland. This cultivar is primarily used for fruit production, b also suitable for vegetable production, and since it is spineless, the mature cladodes are valuable as livestock feed (Cervantes-Herrera et al. 2006). Therefore, this research was conducted to explore the forage quality status of this *D. coccus* resistant cultivar.

2. Materials and methods

The experiment consisted of three *O. ficus-indica* cultivars ('Rojo Pelón', 'Atlixco', and 'Chicomostoc'). 'Rojo Pelón' is resistant to *D. coccus* whereas the others are susceptible (Berhe et al. 2022). The plants were grown at the field of the University of Guadalajara, Center of Biological and Agricultural Sciences (CUCBA), located in the Las Agujas Nextipac, Zapopan, Jalisco, Mexico. Its geographic coordinates are 20º43 North latitude and 103º23 North longitude. The west altitude of 1650 m, with an average annual temperature of 18º C. The soils are of acidic pH and fine sandy texture. Cladodes were planted raw with 50 cm plant spacing and 1 m raw spacing and samples were taken on May 2022.

The experimentation was set in a completely randomized design (CRD) with three replications. One year old cladodes were collected andwere dried in the ventilated oven for 72 hours at 60 ºC for the Bromatological determination of protein, fiber, fat, and minerals. The dried samples were crushed with a juice maker.

Determination of crude protein

The determination of crude protein was done using the Kjeldhl method (Bradstreet 1954). From the powder, 0.7 g was taken. Copper sulphate (7 g) and potassium sulphate (0.8 g) were measured and mixed with the sample. Samples were added to 10 mL of sulphuric acid and put in heating digestion at 420 ºC for an hour. Then the solution was distilled with 30 mL of 4% bromic acid, and 35% of sodium hydroxide and titrated with 0.2 M of hydrochloric acid. Quantity was calculated based on the amount of HCL used to colour change. The nitrogen content and hence the crude protein content were calculated using the formula below. 1 of NH2SO4 = 14 mg protein (%) N2 (%) × 6.25 $N2$ (%) = $100 * N14Vt * T.\ B\ W * 1000 * VA$ Where W= Weight of sample of sample (0.5g); N= Normality of titrant (0.02N H2S04); Vt= total digest volume (100 mL); T= sample titre value; B= blank titre value (Ilodibia et al. 2014).

Determination of fats

The extraction of fats was done with the Soxhlet method (De Castro & Priego-Capote 2010). Two grams of powder samples were prepared. And extraction was used for three hours, then precipitation and heating. The dry weight was measured and the fat content was calculated as follows,

% of ethereal extract = (P-p /M) 100
Where:
P = weight in grams of the flask with fat
p = weight in grams of the flask without fat and
M = weight in grams of the sample
% fats as ether extract = N / P x 100

Determination of fibers

According to the methodology of AOAC (1990), two g of the powder sample was boiled with 1.25% sulfuric acid for 30 min. Then the samples were filtered with a sieve. And, transferred the residue to the beaker containing 150 mL of boiling 1.25% NaOH. Detached the glass from the condenser and filtered through the linen cloth. With the help of a very fine spatula, introduced the crucible to the drying oven at 550º C for 30 min and removed the crucible from the muffle and placed it in a desiccator until it cools down. Samples were weighed and crude fiber was calculated as follows

Crude fiber (p/p) (%) = n / P x 100
Where:
N = Grams of residue once the weight of the ashes has been removed
P = Grams of the sample.

Determination of antioxidants, vitamins, phenolics, and flavonoids

One gram floor sample was prepared and dissolved with 500 µL purified water and 500 µL of ether alcohol for 24 hours.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity test was determined by the methodology described by Randhir & Shetty (2007) with modifications (Pham et al. 2022). For each concentration of the curve and sample, three repetitions were performed. 50 µL of each sample and concentration of the gallic acid line were mixed with 2.95 mL of DPPH·60 µM solution dissolved in methanol, proceeded to homogenize, and kept in incubation for 30 min in the dark. Finally, a reading was taken at an absorbance of (Abs) 517 nm. With the absorbance values, the percentage of inhibition was determined by means of the following equation:

Inhibition (%) = (Abscontrol-Abssample)/Abscontrol] x 100
(Marinova & Batchvarov, 2011).

The gallic acid equivalent (EGA) values (mg/mL) of the samples were obtained with the inhibition averages (%) and interpolated in the calibration curve. The results were expressed as a percentage of inhibition per mg EGA.

The content of ascorbic acid in the extracts was determined according to Yen et al. (1996) using the 2,6-dichloroindophenol (DIP) method with modifications. For each concentration of the curve and sample, three repetitions were performed. An extraction solution composed of 1% metaphosphoric acid was prepared. Each of the samples was mixed in a 1:1 ratio with extracting solution and subsequently filtered with filter paper. 200 µL of each sample (diluted 1:1) and concentration of the ascorbic acid line were mixed with 1800 µL of DIP·50 µM solution, left to incubate at room temperature for 15 s, and absorbance at 515 nm was measured. The values of the samples were calculated from the absorbance averages obtained and interpolated in the calibration curve. The results are expressed in mg to ascorbic acid equivalent (AAE)/mL (Martínez-Flores et al 2021).

Total flavonoids were determined according to the method described by Liu et al. (2002). Three repetitions were performed for each concentration of the curve and sample. Calibration curve: a Quercetin (Q) curve from 0 to 0.600 mg was constructed from a stock solution of 1 mg/mL diluted in Ethanol. Determination in samples: 150 µL of each concentration for the curve or sample was mixed with 150 µL of sodium nitrite (NaNO2; 5%), then 150 µL of aluminum chloride (AlCl3; 10%) and 1 mL of sodium hydroxide (NaOH; 1 M). Finally, the mixture was homogenized and a reading was taken at an absorbance of 510 nm. Interpretation: The values of the samples were calculated from the absorbance averages obtained and interpolated in the calibration curve. The results were expressed in mg equivalents to Q (mgEQ/mL) (Martínez-Flores et al 2021).

Determination of humidity

Two g fresh samples were taken and put in a weighted capsules dish and dried in an oven for three hours at 120 ºC. Samples together with capsules were measured. The percentage of humidity was calculated as follows:

Humidity = (Pm – Ps)/m x 100

Where:

Pm = weight of the capsule and fresh sample in g.

Ps = weight of the capsule and dried sample in g.

m = weight of fresh sample in g.

In vitro dry matter digestibility

It was determined by the method proposed by Zaklouta et al. (2011) and Goering and Van Soest (1970). The rumen fluid was collected from municipal butchery from a slaughtered healthy beef steer; the rumen fluid was immediately transported in pre-warmed bottles to the laboratory. The fluid was filtered through two layers of cotton gauze into a flask while passing CO2 to the flask to displace air from above the fluid and kept in a warm thermostatic bath at 39 °C. The culture medium was prepared according to the indications from Menke and Steingass (1988). Samples of approximately 1 g were placed into previously weighted ANKOM™ bags. The fermentations were conducted in a 500 mL Erlenmeyer flask in which nine bags were placed, and the ratio of rumen fluid and culture were mixed in a proportion 1:1 v/v and closed with rubber stoppers for 72 hours. Then the flasks were opened and the bags were rinsed with distilled water and placed into an oven for drying at 60°C for 24h. Once the filtration process was finished, the data obtained were processed with the formula proposed by Bacon and Gordon (1980), to determine the *in vitro* dry matter digestibility:

IVMD (%) = ((Weight of dry sample-(Weight of residue-Weight of empty bag)) x100)/ (Weight of dry sample)

Where:

IVDMD = *In vitro* dry matter digestibility.

X-ray analysis of calcium crystals

X-ray diffraction was used to detect the presence of the most important Ca crystalline compounds (Weddellite and Whewellite) (Rojas-Molina et al. 2015). Powder samples were densely packed into an Al frame and put on the X-ray machine. Data was collected from a 2teta scale. Spectrum analysis software (Diffract/AT, Socavin VI-2) was used to extract the data (Rodríguez-García et al. 2007). Graphs developed using the Origin (2022b) software. The collected data were subjected to analysis of variance (ANOVA) using R.4.2.0 statistical package, and descriptive statistics using excel LSD test at (0.05) was applied to compare means among treatments.

3. Results and discussion

3.1 Crude protein content, fat content, crude fiber content, humidity, and *in vitro* dry matter digestibility of three *Opuntia ficus-indica* cultivars

Cultivars showed significant difference in crude protein content (p= 0.0488), fat content (p= 0.0288), and a very high significant difference in crude fiber content (p= 0.0009). Humidity percentage and *in vitro* digestibility were not significantly different among cultivars (p= 0.4340). Crude protein content was higher at 'Atlixco' and 'Chicomostoc', but 'Chicomostoc' had no significant difference from 'Rojo Pelón'. Fat content was higher in the 'Rojo Pelón' cultivar. 'Rojo Pelón' followed by 'Chicomostoc' were superior in crude fiber content (Table 1).

Table 1. *Means of crude protein content, fat content, crude fiber content, humidity, and in vitro dry matter digestibility in the percentage of three Opuntia ficus-indica cultivars.*
Tabla 1. *Medias del contenido de proteína bruta, contenido de grasa, contenido de fibra bruta, humedad y digestibilidad in vitro de la materia seca en el porcentaje de tres cultivares de Opuntia ficus-indica*
in vitro dry matter digestibility in the percentage of three *Opuntia ficus-indica* cultivars.

Cultivar	Crude protein %	Fat %	Crud fiber %	Humidity %	Digestibility %
Atlixco	4.72^{b}	3.13^{a}	4.10^{a}	89.48	68.4
Chicomostoc	4.34^{ab}	3.14^{a}	4.57^{b}	-	70.4
Rojo Pelón	3.62^{a}	4.91^{b}	5.07^{c}	89.96	72.6

Note: Means sharing the same letter are not significantly different.
*Nota:*Las medias que comparten la misma letra no son significativamente diferentes.

Lemos et al. (2017), reported dry matter (93.51), Crude protein (7.06) at *O. ficus-indica* genotypes. Hernández-Urbiola et al. (2010) also found 1.87 g/100 g of fat and 7.07 g/100 g protein at *O. ficus-indica* cultivar. Ayadi et al. (2013) reported 3% of crude protein in *O. ficus-indica.* Pessoa et al. (2020) also reported 4.5% to 4.73% of crude protein in *O. ficus-indica* cultivar. The results of protein content seem to be in the range of previous findings. In contrast, Felker & Bunch (2007) suggested that 4-5% protein in non-fertilized is the average. Santos et al. (2018) also found 4-4.3% of crude protein in three *O. ficus-indica* genotypes. A high dietary crude protein level is ideal for attaining maximum growth and high profitability without causing side effects on the physiology of growing Holstein bulls (Xia et al. 2018). And 6.45% of protein and 2.9% of fat were reported on the 'Rojo Pelón' cultivar (Ortiz et al. 2021), which may be due to ecological, agronomic conditions, cladode drying temperature, extraction methods (Donato et al. 2016; Lemos et al. 2017). Soluble protein content in *D. opuntiae* cultivar was 83% higher than that in the susceptible cultivar tested in one season and no difference in another season (Falcáo et al. 2013). Garcia et al. (2021) noted that morphological productivity and nutritive value varied among resistant cactus genotypes.

The crude fiber content recorded here is lower than the report of Lemos et al. (2017) who reported 9.98% of *O. ficus-indica* cultivars. Low fiber and moderate starch and water-soluble carbohydrate concentrations are desirable characteristics of cactus feed (Batista et al. 2009). The digestible percentage recorded here (68.4%, 70.4%, and 72.6%) are in the range of previous reports. Santos et al. (2018) found 63 to 65% of dry matter *in vitro* digestibility of three *O. ficus-indica* genotypes. Digestibility 71.14%, crude protein 10.76 %, and crude fiber 8% was noted by Tegegne (2001). *In vitro* dry matter digestibility of 74.8 to 82.7% was also reported by Bazie et al. (2019).

3.2 Antioxidants, flavonoids, and ascorbic acid of three *Opuntia ficus-indica* cultivars

Antioxidant capacity measured by DPPH was not significantly different among cultivars (p= 0.599). Cultivars showed a significant difference in flavonoid content (p= 0.0626) and ascorbic acid content (p= 0.0056). The highest flavonoid content (4.98) was recorded by 'Atlixco' followed by 'Chicomostoc' (3.55). Ascorbic acid content was higher in 'Chicomostoc', but statistically similar between 'Atlixco' and 'Rojo Pelón' cultivars. Concurrent with these significant differences among varieties were found in the phenolic concentrations (34.07 to 266.67 mg/kg DM) (Kolniak-Ostek et al., 2020).

Table 2. *Means of DPPH, flavonoids, and ascorbic acid of three Opuntia ficus-indica cultivars.*

Tabla 2. *Medios de DPPH, flavonoides y ácido ascórbico de tres cultivares de Opuntia ficus-indica.*

Cultivar	**DPPH (mgEGA/mL)**	**Flavonoids (mgEQ/mL)**	**Ascorbic acid** (mgEAA/mL)
Atlixco	0.2305	4.9747^{b}	3.9156^{a}
Chicomostoc	0.2352	3.5491^{ab}	4.6468^{b}
Rojo Pelón	0.3460	2.0225^{a}	4.1618^{a}

Note: Means sharing the same letter are not significantly different.
Nota: Las medias que comparten la misma letra no son significativamente diferentes.

3.3 Calcium crystal contents detection of three *Opuntia ficus-indica* cultivars

The XRD analysis showed that the three cultivars synthesized weddellite and whewellite oxalate crystals.

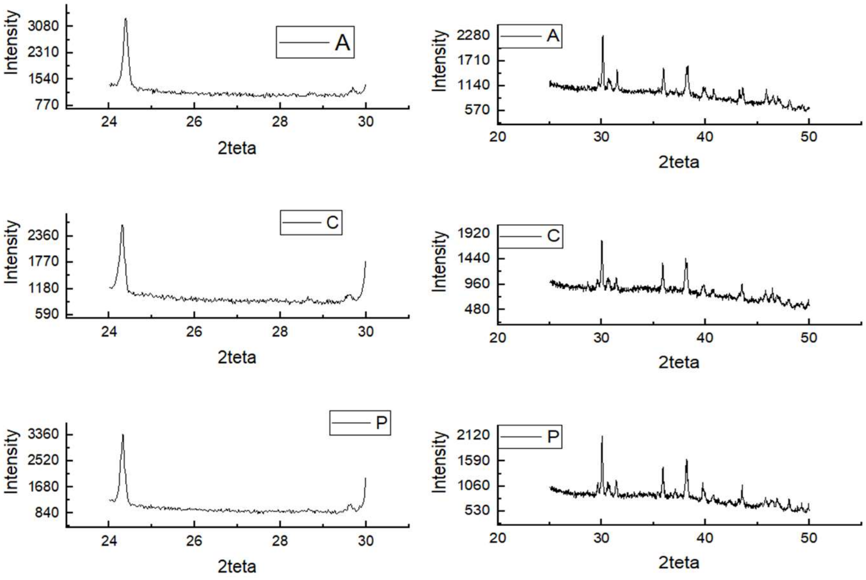

Figure 1. *XRD analysis showed that the three cultivars synthesized weddellite (left) and whewellite (right) oxalate crystals. Weddellite at 2teta of 24.165 and whewellite at 2teta of 24.482. A-Atlixco, C-Chicomostoc and P-Rojo Pelón.*

Figura 1. *El análisis XRD mostró que los tres cultivares sintetizaron cristales de oxalato de weddellita (izquierda) y whewellita (derecha). Weddellita en 2teta de 24.165 y whewellita en 2teta de 24.482. A-Atlixco, C-Chicomostoc y P-Rojo Pelón.*

In plants, calcium oxalate occurs in two hydration states, as monohydrate (whewellite) or as dihydrate (weddellite) (Gadd 1999; Monje & Baren 2005). Both calcium crystals (weddellite and whewellite) were detected in all cultivars. The peak height of weddellite at the X-ray analysis showed that there seem different contents of calcium oxalates at different cultivars. 'Rojo Pelón' seems to have more weddellite crystal as the intensity graph indicates (Figure 1). This is in line with previous reports that calcium oxalate content varies depending on cultivars (Rahman & Kawamura 2011; Dubeux et al. 2021; Tovar-Puente et al. 2007) and resistant cultivars may have more quantity to enhance defense (Mazzeo et al. 2019; Doege 2003; Peschiutta et al. 2020). Calcium oxalate crystals can physical deterrent (Schwachtje & Baldwin; Gish et al. 2016) or are toxic (Prasad & Shivay 2017; Chrigui et al. 2020) to insects. The variation of crystals depending on sites is less than between species; therefore, the value of the crystal characteristics for taxonomy is appropriate (Bárcenas-Argüello et al. 2015). Presence of whewellite in *O. microdasys* (Monje & Baren 1997; Bárcenas Argúello 2015) and in *O. ficus-indica* (Malainine et al. 2003).

Agronomic factors such as growing season, harvesting practices, plant maturity, plant species, plant variety, and plant parts can also have a large effect on oxalate accumulation (Rahman and Kawamura 2011; Duebeux et al. 2021; Li et al. 2022). Cactaceae family could produce diverse biochemical forms of calcium oxalate, signifying a definite but different genetic control (Monje & Baran 2002). Calcium crystals also may have functions in removing excess calcium, magnesium, and sulfur, protecting the plants against herbivory, and detoxifying aluminum and heavy metals (He et al. 2012; Li et al. 2014).

Resistant cultivars can have low dry matter digestibility (Burton et al. 1997; Santo et al. 2017). Nutrition content and palatability are important considerations during pest resistance breeding (Gentile & Malfa 2022) and some *Dactylopius* spp resistant cultivars can have good value (Garcia et al. 2021). The lower protein content in resistant cultivars could be associated with the unsuitability of the insect. The lower the protein content, the lesser the insect performs (Broadway & Duffey 1988; Chiozza et al. 2010; Akbar et al. 2014; Leybourne et al. 2019). Proteins are important nutrition components for the insect for growth and development (Le Gall & Behmer 2014; Behmer 2009). The lower content of flavonoids in the resistant cultivar contradicts some reports that flavonoids have a role in insect defense (Matos et al., 2021; Simmonds 2001; Panche et al. 2016), but literature argues that some flavonoids favor the insect herbivory (Wang et al. 2012; Golawska et al. 2008). Or they can have a neglected effect on defense (Alon et al. 2013).

4. Conclusions

The nutritional quality of the tested resistant cultivar, 'Rojo Pelón' is comparable with the susceptible cultivars. It has lower crude protein content but is superior in fat and fiber content compared to the susceptible cultivars. It has an equivalent nutritional quality like humidity, *in vitro* dry matter digestibility, flavonoid, ascorbic acid, and antioxidant capacity. The important nutritional factors recorded are in the range of the previous research reports on *O. ficus-indica* cultivars. Therefore, in addition to *D. coccus* insect resistance, the cultivar has reasonable forage nutritional importance to be used as forage like other spineless cultivars.

Contribution of the authors in the development of the work: YKB, LPB, and PSRE designed experiments. YKB, perform the experiment. YKB, MADAA, RCC, DVA, and PSRE did the chemical analysis and data management. YKB conducted data analysis and wrote the manuscript draft. YKB, LP, and PSRE did the review and editing. All authors commented on the manuscript and approved the submitted version.

Interest conflict

There is no conflict of interest among authors.

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CAPÍTULO 6. CONCLUSIONES Y RECOMENDACIONES GENERALES

El género Opuntia es originaria de Mesoamérica y está ampliamente distribuida por todo el mundo. La reproducción sexual parece haber contribuido al éxito evolutivo de *Opuntia*, y hubo selección artificial realizada por humanos. *O. ficus-indica* es una especie domesticada y ha sido un cultivo importante en la agricultura y el consumo humano durante miles de años. La domesticación de *Opuntia* spp. ha llevado a una gran diversidad intraespecífica, pero también ha afectado negativamente la capacidad de las plantas para sobrevivir en la naturaleza.

Dactylopius coccus es un parásito de los nopales, principalmente *Opuntia* spp., con diferentes especies de *Dactylopius* prefiriendo también diferentes especies de *Opuntia* como hospederos.

Las interacciones entre insectos y plantas han evolucionado juntos durante millones de años. Las plantas han desarrollado mecanismos de defensa contra los herbívoros, incluida la producción de sustancias químicas defensivas y compuestos orgánicos volátiles en respuesta al daño de los herbívoros. Los insectos, por su parte, han evolucionado para reconocer plantas huésped que producen metabolitos secundarios específicos a través de sus quimiorreceptores finamente sintonizados. La importancia química y ecológica de los metabolitos secundarios de las plantas está relacionada con la vida de los insectos, incluida la búsqueda de huéspedes, las barreras químicas, la defensa, la comunicación sexual y la polinización.

El uso de variedades de cultivos resistentes a plagas es muy importante, lo que puede proporcionar beneficios económicos, ambientales y ecológicos. Tales variedades pueden reducir las pérdidas de cosechas debido a los insectos y minimizar el costo de los insecticidas. También están fácilmente disponibles para los pequeños agricultores, especialmente en los países en desarrollo. Y ayudan a reducir el uso de productos químicos, reduciendo indirectamente el riesgo de contaminación ambiental y problemas de salud humana.

Los cultivares resistentes, 'Robusta' y 'Rojo Pelón', presentaron menor número de ninfas I, y no se observaron ninfas II en estos cultivares. En contraste, los cultivares susceptibles, 'Atlixco', 'Chicomostoc' y 'Nopalea', presentaron mayor número de ninfas I y ninfas II. El resultado mostró que 'Rojo Pelón', que es un cultivar de *O. ficus-indica*, es resistente a *D. coccus*. Además, otra prueba confirma la resistencia de dos genetipos ('Punto 1', 'Punto 2' y 'Sandate 2'). Este estudio puede ser de utilidad para las regiones que tienen problemas de *D. coccus* para uso inmediato y para crianza. El análisis nutricional de este cultivar también confirmó que tiene buena calidad forrajera.

Los cultivares resistentes presentan mayor espesor de epidermis y más cristales de oxalato de calcio en comparación con los cultivares susceptibles. Los cristales de oxalato de calcio sirven para disuadir la alimentación de los insectos y reducen su tasa de crecimiento y aumentan la mortalidad. El estudio también encontró una correlación positiva significativa entre el grosor del cladodio, el número de oxalatos de calcio y el grosor de la epidermis con la resistencia a *D. coccus*. La presencia de cristales de oxalato de calcio y mayor espesor de la epidermis se asociaron con resistencia frente a *D. coccus*.

El análisis de los perfiles de metabolitos de diferentes cultivares de plantas de nopal y cómo estos perfiles se relacionan con su resistencia a la herbivoría de insectos demostró que los cultivares resistentes tenían concentraciones más altas de metabolitos secundarios, que podrían actuar como disuasivos de alimentación o toxinas para los insectos. Algunos metabolitos fueron específicos de ciertos cultivares y se correlacionaron con respuestas de herbivoría específicas.

Con base en los resultados del estudio, se puede concluir que los cambios metabólicos ocurren entre los cultivares de nopal asociados con la resistencia al insecto *D. coccus*. La variación de metabolitos en diferentes cultivares de cactus sugiere que puede haber metabolitos únicos asociados con la resistencia a insectos que podrían usarse para programas de fitomejoramiento. Los perfiles metabólicos variaron entre las diferentes etapas de crecimiento de la planta y pueden estar relacionados con la resistencia intrínseca e inductiva de la planta a los herbívoros. Comprender las moléculas de señal, su modo de acción y la transducción de señales en las interacciones planta-insecto es crucial para desarrollar estrategias efectivas de manejo de plagas.