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EVALUACIÓN DE TRATAMIENTOS POSCOSECHA CON SILICIO PARA EL CONTROL DE LA ANTRACNOSIS (*Colletotrichum brevisporum*) EN PAPAYA MARADOL

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EVALUACIÓN DE TRATAMIENTOS POSCOSECHA CON SILICIO PARA EL CONTROL DE LA ANTRACNOSIS (*Colletotrichum brevisporum*) EN PAPAYA MARADOL

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Colegio de Postgraduados, 2021

RESUMEN

Se reporta por primera vez en este estudio, el hongo *C. brevisporum* causando la antracnosis en frutos de papaya Maradol en México (Capítulo III). Asimismo, se determinó el efecto antifúngico del Silicio (Si) *in vitro* sobre *C. brevisporum* e *in vivo* sobre la antracnosis poscosecha de papaya Maradol (Capítulo IV). Las concentraciones de 3.0 y 2.5% de silicato de potasio (PS) y silicato de sodio (SS) fueron las más efectivas para reducir significativamente el crecimiento micelial del hongo con 93 y 91%, y 100 y 100% de inhibición de crecimiento micelial de *C. brevisporum*, respectivamente. El Si no tuvo efecto sobre la antracnosis en frutos de papaya inoculadas artificialmente con *C. brevisporum* en tratamientos curativos primarios *in vivo* durante 6 d de incubación. Solamente el PS al 1.5% previno significativamente la incidencia de la antracnosis (6.3% de incidencia), pero no la severidad; mientras el SS al 1.0% suprimió totalmente la incidencia de la antracnosis, y además redujo la severidad. Además, se encontró que los mejores tiempos de baño para PS al 1.5% y SS al 1.0% fueron 60 y 90 s, con valores de incidencia de 6.7 y 0%, respectivamente, en tratamientos preventivos durante 5 d. Finalmente, los tratamientos preventivos combinados de Si con TBZ no tuvieron efecto sobre la antracnosis después de 5 d de incubación.

Palabras claves: *Colletotrichum brevisporum*, antracnosis, incidencia, severidad**.**

EVALUATION OF POST-HARVEST TREATMENTS WITH SILICON FOR THE CONTROL OF ANTHRACNOSIS (*Colletotrichum brevisporum*) IN PAPAYA MARADOL

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Colegio de Postgraduados, 2021

SUMMARY

We are reporting the fungus *C. brevisporum* by first time causing anthracnose on Maradol papaya fruit in Mexico (Chapter III). Likewise, the antifungal effect of silicon was determined *in vitro* on *C. brevisporum* and *in vivo* on the postharvest anthracnose of papaya cv. Maradol (Chapter IV). The concentrations of 3.0 and 2.5% of potassium silicate (PS) and sodium silicate (SS) were the most effective treatments to significantly reduce the mycelial growth of the fungus, with values of 93 and 91%, and 100 and 100% of inhibition of mycelial growth of *C. brevisporum*, respectively. Silico had no significant effect on anthracnose disease on the papaya fruit, artificially inoculated with *C. brevisporum*, in *in vivo* primary screenings curative treatments for 6 d of incubation. Moreover, only 1.5% PS significantly prevent the incidence of anthracnose disease (6.3% incidence), but not the severity; while 1.0% SS totally suppressed the incidence of anthracnose decay. Also it was found that the best time for 1.5% PS and 1.0% SS in aqueous solutions was an immersion time of 60 and 90 s, with incidence values of 6.7 and 0%, respectively, in preventive treatment for 5 d of incubation. Finally, the preventive treatments of Si combined with TBZ did not significantly improve the control of postharvest anthracnose disease on Maradol papaya after 5 d of incubation at 25-26°C.

Keywords: *Colletotrichum brevisporum*, anthracnose, incidence, severity.

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CAPÍTULO I. INTRODUCCIÓN GENERAL

La papaya (*Carica papaya L.*) pertenece a la familia *Caricaceae*, originaria de Mesoamérica y América central (Singh *et al*., 2010), fue descrita por primera vez en 1526 por el cronista español Oviedo, quien la encontró en las costas atlánticas de Panamá y Colombia (SAGARPA, 2005). Da acuerdo a Morton (1987), este cultivo se dispersó a otros continentes después de la colonización de América por los españoles; existen reportes del transporte de la semilla en el año 1550 de Panamá y República Dominicana a Norteamérica y Sudamérica. En ese mismo siglo los marinos portugueses llevaron la semilla a Malasia e India (Hill y Waller, 1988). Para 1600 aproximadamente, estaban cultivando papayas en regiones cálidas de Sur y Centro América, las Antillas, Bahamas, Bermudas Florida y Sur de México (SAGARPA, 2005).

La papaya es un fruto ampliamente consumido en México; en 1978 fue introducida al país la variedad Maradol, que es la más comercial a nivel nacional (SAGARPA, 2005). En 2019, México ocupó el cuarto lugar en producción de frutos de papaya con 1083133 ton (en una superficie de 18839 ha) a nivel mundial, después de India, República Dominicana y Brasil (FAO, 2021).

En 2019 Tabasco representó el tercer lugar en rendimiento de papaya Maradol (78.72 ton ha^{-1}) a nivel nacional, y ocupó el 13º lugar en producción de papaya Maradol (7951 toneladas, ton) a nivel nacional (SIAP, 2020). Las pérdidas de papaya en la poscosecha son debido a varios factores, tales como daños mecánicos, daños por frío, senescencia del fruto y enfermedades.

Salunkhe y Desai (1984) estimaron que las pérdidas de papaya en poscosecha varían de 40 a 100% dependiendo de la zona de producción en el mundo. Singh (2010) menciona que las

enfermedades poscosecha causadas por hongos son los problemas más importantes durante el manejo y almacenamiento de la papaya; y dentro de ellas, la antracnosis en frutos, es la enfermedad poscosecha más importante, con pérdidas de alrededor de 25-50% en India y Estados Unidos (Singh, 2010; Maeda y Nelson, 2014).

En países como Estados Unidos (Dickman y Álvarez, 1983; Paull *et al.*, 1997), Malasia (Ali, 2008), México (Casarrubias-Carrillo *et al*., 2002; Bautista-Baños *et al*., 2003), la antracnosis de la papaya se había asociado principalmente al hongo *Colletotrichum gloeosporioides*, cuya infección causa lesiones húmedas, hundidas y con masas de conidios de color naranja. En la última década, se han reportado que varias especies del genero *Colletotrichum* (Cuadro 1.1) están asociadas a la antracnosis en papaya. En el 2017, Lira-Vargas *et al*. (2017) y Torres-Calzada *et al*. (2013) reportan varias especies de *Colletotrichum* aislados de frutos de papaya en México (Cuadro 1.2).

El control del agente causal de la antracnosis está basado principalmente en el uso de fungicidas químicos sintéticos convencionales tales como tiabendazol, benomil, y procloraz. Sin embargo, el uso de estos productos trae problemas de acumulación de residuos en el fruto de papaya, contaminación en el medio ambiente y resistencia del hongo a estos fungicidas. Bajo este contexto, esta investigación de tesis tuvo como propósito, buscar un control no contaminante alternativo al control convencional, que coadyuve a encarar la problemática de la enfermedad de la antracnosis en frutos de papaya mediante el uso de productos químicos más amigables con el medio ambiente y la salud humana tal como el silicio (Si) en sus formas de silicato de potasio (Ag Sil® 21 PQ Corporation, PS) y silicato de sodio (sodium silicate, 1.4 g cm^3 PQ Corporation, SS). Además, a nuestro conocimiento, no hay reportes de

investigación sobre la actividad antifúngica del Si en la antracnosis específicamente en el fruto de papaya.

Cuadro 1.1. Especies de *Colletotrichum* asociadas a la antracnosis en frutos de papaya a nivel mundial.

Especie	**País**	**Referencia**
C. truncatum	Trinidad	Rampersad, 2011
	México	Torres-Calzada *et al*., 2013, 2018 Rojo-Báez *et al*., 2017 De la Rosa-García *et al*., 2018
	República de Corea	Aktaruzzaman *et al.*, 2017
	Brasil	dos Santos-Vieria, 2020
C. capsici	Malasia	Rahman *et al*., 2008
C. fructicola	India	Saini *et al.*, 2016
	Ecuador	Vilaplana *et al*., 2020
C. salsolae	India	Saini *et al.*, 2017
C. gloeosporioides	Costa Rica	Solano y Arauz, 1995
	Sri Lanka	Gamagae *et al*., 2004
	Trinidad	Rampersad, 2011
	Estados Unidos	Maeda y Nelson, 2014
	Malasia	Rahman *et al*., 2008; Ong y Ali, 2015
	México	Rojo-Báez *et al*., 2017; De la Rosa-García *et al*., 2018
C. brevisporum	Brasil	Viera *et al*., 2013
	Australia	Shivas *et al*., 2016
	México	Lira-Vargas *et al*., 2017
	Taiwán	Duan *et al*., 2018

Cuadro 1.2. Presencia y distribución de especies de *Colletotrichum* aislados de frutos de papaya en México.

Especie	Estado	Referencia
C. brevisporum	Campeche Chiapas (Acapetahua, Mazatán) Colima (Centro y Tecomán) Guerrero (La Unión de Isidro) Jalisco (Tomatlán) Michoacán (Coahuayana) Oaxaca (Villa de Tututupec de Melchor Ocampo, Pinotepa Nacional, Santiago Jamiltepec) Tabasco (Balancán) Yucatán (Tizimín) Veracruz (Cotaxtla, Soledad de Doblado)	Lira-Vargas *et al*. (2017) Torres-Calzada *et al*. (2013)
C. cliviae	Campeche (Halcechakan) Chiapas (Tapachula) Oaxaca (Santa María Jalapa de Marqués) Veracruz (Tierra blanca)	Lira-Vargas *et al*. (2017) Torres-Calzada *et al*. (2013)
C. truncatum	Colima (Centro)	Lira-Vargas *et al*. (2017)
C. gloeosporioides	Guerrero (La Unión de Isidro) Veracruz (Tlalixcoyan, Soledad de Doblado) Quintana Roo (Morelos, Quizás)	Lira-Vargas *et al*. (2017) Torres-Calzada *et al*. (2013)
C. karstii	Michoacán (La Huacana)	Lira-Vargas *et al*. (2017)
C. aenigma	Veracruz (Actopan)	Lira-Vargas *et al*. (2017)
C. capsici	Chiapas (Tapachula) Quintana Roo (Morelos, Quizás) Campeche (Champotón)	Lira-Vargas *et al*. (2017) Torres-Calzada *et al*. (2013)

1.1. Objetivos

Objetivo general:

Determinar el efecto antifúngico del silicio en sus formas de silicato de potasio y silicato de sodio sobre la antracnosis en Papaya Maradol en poscosecha.

Objetivos particulares:

1. Aislar e identificar el hongo causante de la antracnosis en frutos de papaya.
2. Determinar el efecto *in vitro* del silicio sobre el crecimiento micelial de *Colletotrichum brevisporum*.
3. Evaluar el efecto *in vivo* del silicio sobre la antracnosis (*C. brevisporum*) mediante experimentos primarios.
4. Determinar la influencia del tiempo de inmersión en la efectividad del silicio sobre la antracnosis (*C. brevisporum*).
5. Evaluar el efecto del silicio solo o combinado con dosis bajas de tiabendazol sobre la antracnosis (*C. brevisporum*).

1.2. Hipótesis

El silicio en sus formas de silicato de potasio (PS) y silicato de sodio (SS) inhiben el crecimiento micelial y tiene un efectivo control sobre la antracnosis causada por *Colletotrichum brevisporum* en poscosecha de frutos de papaya.

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CAPÍTULO II. REVISIÓN DE LITERATURA

2.1. Antracnosis

Dentro de las enfermedades poscosecha que afectan a la papaya se encuentra la antracnosis como la más importante (Bautista-Baños *et al*., 2013), provocada por el patógeno *Colletotrichum gloeosporioides* (Ong y Ali, 2015).

Esta enfermedad se caracteriza por presentar lesiones oscuras y hundidas, circulares elipsoidales, con grandes cantidades de esporas formando masas compactas de color salmón, naranja o rosadas (Coria, 2009).

El fruto desde su desarrollo en la planta y hasta su poscosecha, sufre daños por *C. gloeosporioides*, ocasionando pérdidas hasta de 100%, dependiendo de las condiciones climáticas que prevalezcan en una región (Landero-Valenzuela *et al*., 2016).

Algunos de los factores que determinan los daños económicos son: la densidad de inóculo del patógeno, el manejo preventivo en campo que se efectúa para reducir su impacto y de las exigencias del mercado al cual está destinado la fruta (Ventura *et al*., 2006).

2.1.1. Síntomas

El hongo puede penetrar directamente la cutícula del fruto mediante los apresorios o através de los estomas y heridas en la corteza del fruto, cuando el fruto inicia el proceso de maduración después de 30 h de la inoculación (Ayón-Reyna *et al*., 2017; Rojo-Baéz et al., 2017). Las hifas intramurales crecen en las paredes celulares de células epidérmicas a las 30-48 h después de la inoculación; la colonización necrotrófica inicia a las 72 h después de la inoculación. Finalmente, hifas intracelulares crecen dentro de las células del parénquima,

causando una extensiva degradación celular, lo cual ocasiona que se formen lesiones típicas de antracnosis (Durán y Mora, 1987; Barqueros-Quirós *et al*., 2013; Rojo-Baéz *et al*., 2017) (Figura 2.1.). Estas lesiones aumentan de tamaño hasta observarse lesiones húmedas y colonias de esporas de color naranja o rosado en la parte central de la lesión (Citlali *et al*., 2005). En Brasil, se ha reportado que la pulpa puede tener un detrimento en el sabor de la misma en daños severos (Texeria da Silva *et al.*, 2007).

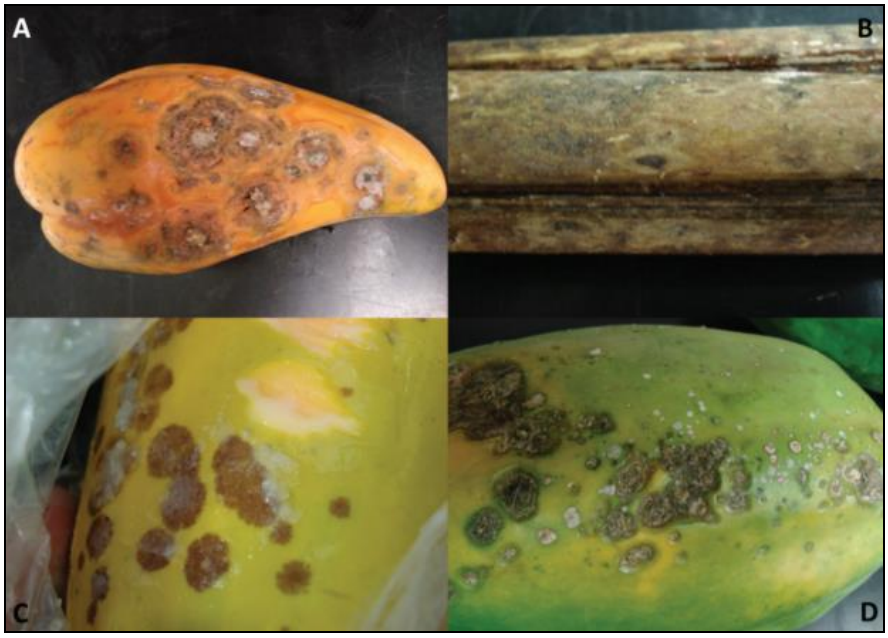

Figura 2.1. Síntomas típicos de antracnosis causados por *Colletotrichum* spp., en papaya. (A) Fruto maduro. (B) Pecíolo. (C) Mancha chocolate. (D) Fruto inmaduro (Barqueros-Quirós *et al.*, 2013).

2.1.2. Ciclo de la enfermedad

Las condiciones ambientales que favorecen al patógeno son las temperaturas entre 18-25°C y humedad relativa de 97% como mínimo (Garza-López *et al*., 2001).

La fuente de inóculo primario más importante, lo constituyen los frutos infectados no removidos y caídos (Ploetz, 1979); este inóculo es diseminado por el viento, la lluvia o insectos y afectan inicialmente en el campo a los pecíolos y hojas viejas (Calzada, 1975), de donde se propaga a los frutos inmaduros, sanos y sin heridas (Mont, 1998).

Las especies de *Colletotrichum* utilizan dos estrategias principales de infección: colonización hemibiotrófica intracelular o colonización intramural subcuticular.

El estado inicial de infección es muy similar en ambas estrategias: el conidio se adhiere sobre la superficie del fruto, produce el tubo germinativo y luego en su extremo forma el apresorio melanizado con el cual penetra directamente la cutícula; sin embargo, el proceso de colonización presenta diferencias en cada estrategia (Perfect *et al*., 1999). Durante la colonización hemibiotrófica intracelular, las hifas aumentan de tamaño al penetrar las células epidérmicas del hospedante, formando vesículas de infección y alrededor de la hifa primaria amplia, se invagina la membrana plasmática. La vesícula de infección y la hifa primaria están rodeadas por una matriz interfacial, por lo que el protoplasto del hospedante se mantiene vivo durante esta etapa de la interacción; posteriormente, la hifa primaria coloniza progresivamente nuevas células epidérmicas y mesófilas, a partir de las hifas primarias se forman hifas delgadas secundarias necrotróficas , las cuales se expanden rápidamente y degradan las paredes celulares mediante digestión enzimática, lo que ocasionan lesiones necróticas (Perfect *et al.*, 1999).

En contraste, durante la estrategia de infección intramural subcuticular, después de la penetración, el patógeno se desarrolla debajo de la cutícula formando una red de hifas intramurales. Durante las etapas posteriores de infección, las hifas inter e intracelulares

penetran células epidérmicas y mesófilas, lo que ocasiona la muerte del hospedante (Perfect *et al*., 1999).

Se requieren de aproximadamente de 48 h para que ocurra la germinación de los conidios sobre la cutícula de la fruta y de 72 h para que suceda la penetración del patógeno a través de la misma (Figura 2.2).

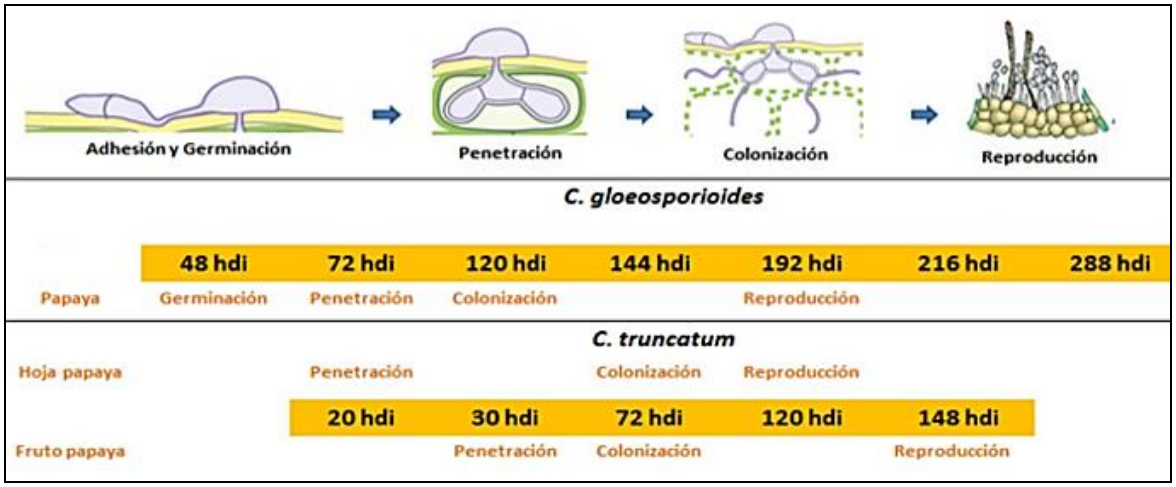

Figura 2.2. Etapas del proceso de infección de *Colletotrichum* spp. reportadas en México (Rojo-Báez *et al*., 2017).

A partir de las 120 h después de la inoculación (hdi), se puede observar la existencia de hifas en los tejidos del epicarpo y del mesocarpo. También se aprecia la formación de cavidades por debajo de las zonas afectadas por el patógeno, producto de la degradación de las paredes celulares. Luego de 8 d de inoculación, las diferentes capas celulares se encuentran invadidas por el hongo, acompañado de la generación de acérvulos (Casarrubias-Carrillo *et al*., 2002).

Las infecciones de *C. gloeosporioides* ocurren principalmente en el campo durante el desarrollo de las frutas, que puede darse entre las cuatro a catorce semanas posteriores a la antesis floral (Dickman y Alvarez, 1983). Estas se mantienen latentes, en forma de apresorio sin germinar (Jeffries *et al*., 1990) hasta que el fruto entra en su fase climatérica, donde aparecen los primeros síntomas de la enfermedad (Casarrubias-Carrillo *et al*., 2002). Al avanzar la maduración del fruto, se da la exudación de gotas de látex sobre la superficie de la fruta, las cuales favorecen al desarrollo de las lesiones húmedas (Álvarez y Nishijima, 1987).

2.1.3. Control de la antracnosis con sustancias de baja toxicidad

Varios trabajos de investigación han demostrado el potencial de sustancias químicas de baja toxicidad o más amigable con el medio ambiente para el control de la antracnosis en papaya. Por ejemplo, el extracto de raíces de *Gliricida sepium* contra *C. gloeosporioides* fue probado reduciendo la severidad de la antracnosis 94% sobre frutos de papaya Hawaiana en poscosecha (Loaiza y Rivera, 2000).

Sivakumar *et al*. (2002) encontraron que el carbonato de amonio (3%) incorporado en la formulación de cera redujo un 70% la incidencia de antracnosis en papaya infectada naturalmente.

Por su parte Bautista-Baños *et al*. (2003), evaluaron el efecto fungicida *in vitro* del quitosano, encontrando que las concentraciones al 2.0 y 3% suprimieron completamente el crecimiento micelial de *C. gloeosporioides* y estos mismos autores reportaron un control de la antracnosis (40% de incidencia) con quitosano a concentraciones de 1.5% después de 7 d de incubación en experimentos *in vivo*. Ali (2008) encontró que el quitosano a las concentraciones de 2.0 y

1.75% inhibieron el crecimiento micelial de hasta 100 y 94%, respectivamente, así como también la inhibición de germinación de conidios de 100 y 89%, respectivamente de *C. gloeosporioides*.

En otro trabajo de investigación, Siqueira-Júnior *et al*. (2012), investigaron el potencial del aceite de ricino para el control de la antracnosis en papaya y encontraron que el aceite de ricino a concentraciones de 5 y 10% redujeron significativamente el crecimiento micelial y la esporulación de *C. gloeosporioides*, respectivamente.

Landero-Valenzuela *et al.* (2013) demostraron que el aceite de canela a concentraciones de 0.0015, 0.0025 y 0.005% tuvieron un mejor efecto de inhibición de crecimiento micelial, aunque el extracto de ajo a concentración de 10% inhibió al 100% el hongo de *C. gloeosporioides*.

Por su parte, Ong y Ali (2015) encontraron que el ozono aplicado a 3.5 y 5.0 µl L^{-1} degradaron la mitocondria en las esporas de *C. gloeosporioides* tras 24 h después del tratamiento.

Investigaciones más recientes también han evidenciado el control de la antracnosis en papaya. Por ejemplo, Ayón-Reyna *et al*. (2017) reportaron que el efecto combinado del tratamiento hidrotérmico-cloruro de calcio redujeron la incidencia y severidad de la antracnosis en comparación con el control, durante 10 días de almacenamiento de papaya a 12°C.

Asimismo, Ferreira *et al*. (2017) revelaron que usando la levadura (*Anthracocystis*

grodzinskae) UFT 5852 y el bicarbonato de sodio solos o combinados redujeron la severidad de la enfermedad en un 93.7, 100 y 84.4%, respectivamente en experimentos *in vivo* en papaya.

De La Rosa-García *et al*. (2018), evaluaron la actividad antifúngica de nanopartículas de óxidos de metales sobre cepas de *Colletotrichum gloeosporioides* encontradas en frutos de papaya y aguacate; los resultados revelaron que las nanopartículas de óxido de zinc (ZnO) son un poderoso agente antifúngico contra dicho patógeno, obteniendo control sobre la antracnosis en ambos frutos tropicales.

Maringgal *et al*. (2020) reportaron que las nanopartículas de óxido de calcio (CaO) muestran alta actividad antifúngica, obteniendo la mejor respuesta en concentraciones del 15% en contra de la antracnosis de frutos de papaya causada por C*olletotrichum brevisporum*.

2.2. El silicio

El Silicio (Si) es el segundo elemento más abundante en la corteza terrestre, únicamente superado por el oxígeno y aunque no es considerado un nutriente esencial para los vegetales, es conocido que mejora la tolerancia de éstas a la sequía (reduciendo la transpiración), le aumenta la resistencia al estrés salino (Liang *et al*., 2008) y reduce la intensidad de daños de plagas y enfermedades que las plantas atacan (Malavolta, 2006; Datnoff *et al*., 2007; Kaluwa *et al*., 2010; Pozza *et al.*, 2015; Fauteux *et al.*, 2005). El Si no se encuentra libre en la naturaleza, sino se encuentra en formas combinadas, ya sea como la sílice y minerales siliconados (Castellanos-González *et al*., 2015).

Además, se considera que juega un papel fundamental como un componente de las paredes celulares (Laing *et al*., 2006).

2.2.1. Antecedentes del silicio en la agricultura

En la agricultura, compuestos con Si se ha usado como fertilizantes y para el control de plagas y enfermedades, los primeros trabajos sobre el papel que juega el Si en el control de enfermedades de las plantas se iniciaron desde la década de 1920. En 1991, O'Neill demostró que la adición de silicato de potasio (K_2SiO_3) a soluciones de nutrientes reducía la incidencia de mildiu polvoriento (*Pseudoperonospora cubensis*) y las lesiones en tallo causadas por Didymella bryoniae y *Botrytis cinerea* en plantas de pepino (*Cucumis sativus*).

La aplicación del silicio juega un papel importante en el crecimiento y desarrollo de las plantas, incluida la polinización enriquecida, el aumento de la biomasa seca y el rendimiento (Korndörfer y Lepsch, 2001).

Agarie *et al* (1998) reportaron el potencial que tiene el silicio (como nutriente en solución) para reducir la pérdida de electrolitos en hojas de arroz; cuando aplicaron 120 mg g^{-1} de silicio (en forma de SiO_2) registraron un valor de 57 % de pérdida de electrolito menor al valor obtenido en el testigo (67 % de pérdida de electrolito).

Por su parte, Dann y Muir (2002) estudiaron el efecto del silicato de potasio como fertilizante en plántulas de chicharos (*Pisum sativum* cv. Alaska or Greenfea), obteniendo como resultado que el silicio incrementa la actividad de enzimas de defensa (quitinasa y β-1,3-glucanasa).

Kaluwa *et al*. (2010), estudiaron el efecto de aplicación del silicio sobre el fruto de aguacate "Hass", encontrando que este elemento pasa a través del tejido del exocarpo al mesocarpo, cuando se aplican altas concentraciones de silicio (silicato de potasio, KSil 2940 ppm), siendo benéfico para mantener la calidad del fruto de aguacate (*Persea americana* Mill. cv. 'Hass'), probablemente debido a la supresión de la respiración y reducción de producción de etileno.

Mditshwa *et al*. (2013) mencionan que las inmersiones de 30 minutos con silicato de potasio (K_2SiO_3) a 50 mg L^{-1} redujeron la aparición de síntomas de lesión por frío en limones "Eureka" (*Citrus limon*) en poscosecha, mientras que concentraciones más altas aumentaron el daño por frio. De acuerdo a los ensayos que Markovich *et al.* (2017) realizaron con arabidiopsis (*Arabidopsis thaliana* var. Columbia salvaje) y sorgo (*Sorghum bicolor*), aseveran que el silicio promueve la biosíntesis de citoquininas y retrasa la senescencia de los tejidos en la totalidad de la planta, aumentando la durabilidad tanto de hojas como de fruta.

2.2.2. Control de enfermedades con silicio

En el 2005, Smith *et al.* demostraron que aplicaciones de silicato de potasio en la irrigación redujeron considerablemente la marchitez causada por *Fusarium oxysporum* f. sp. *vasinfectum* en algodón (*Gossypium barbadense*). Por su parte, Anderson *et al*. (2005) encontraron que inyecciones de silicio soluble (1000 ppm y 2000 ppm) en árboles de aguacate (*Persea americana* Mill. cv. 'Hass') antes de la cosecha disminuyó significativamente la severidad e incidencia de la antracnosis.

Guo *et al*. (2007) proponen que el silicato de sodio ($Na_2O{\cdot}nSiO_2$) a concentraciones de 100 mM estimula los sistemas naturales de defensa de las plantas a través de la producción de compuestos fenólicos y activación de las enzimas peroxidasa (POD) y fenilalanina amonio liasa (PAL) en frutos de melón (*Cucumis melo* L.). Además, David y Weerahewa (2012) concluyeron que el silicio aplicado a 50mg L^{-1} o 100mg L^{-1} a la planta de tomate (*Lycopersicon esculentum* L.) en las etapas fenológicas de crecimiento, floración o ambos estados maduros tiene un efecto significante en reducir el desarrollo de la antracnosis (*C. gloeosporioides*) en frutos de tomate.

Polanco *et al*. (2014) mencionó que las concentraciones de silicio (2 mM Si^{+}) adicionadas como ácido monosílico a la solución de nutrientes adicionada a la planta de frijol (*Phaseolus vulgaris* L. cv. Pérola), redujo la severidad de la antracnosis (*Colletotrichum lindemuthianum*) por 34%. En estudios más recientes, Van Bockhaven *et al.* (2015) estudiaron el transcriptoma de las plantas de arroz (*Oryza sativa* cv Nipponbare) de control y plantas tratadas con silicio (2mM ácido silícico) infectadas con el hongo necrotrófico de la mancha marrón C*ochliobolus miyabeanus*, encontrando que el silicio mejora la resistencia a la mancha marrón al contrarrestar la senescencia inducida por *C. miyabeanus* y la muerte celular, a través del aumento de la fotorrespiración. Asimismo, Wang *et al* (2020) estudiaron el silicio en la planta arabidopsis (*Arabidopsis thaliana* salvaje), sugiriendo que el silicio asimilado modula distintos mecanismos de defensa de múltiples capas para mejorar la resistencia de la planta contra patógenos adaptados o no adaptados del mildiú polvoroso (*Golovinomyces cichoracearum* UCSC1), posiblemente a través de la interacción sinérgica con la defensa-inducida de producción de callosa.

2.2.3. Control de enfermedades poscosecha con silicio

Tratamientos poscosecha de silicio en la forma de silicato de sodio mostraron control contra *A. alternata*, *Fusarium semitectum* y *Trichothecium roseum* en melones (Bi *et al*., 2006; Guo *et al*., 2007). Asimismo, Ligorio *et al*. (2007) encontró que el silicato de sodio controló las podredumbres verde (*Penicillum digitatum*) y azul (*Penicillum italicum*) en mandarinas clementinas (*Citrus clementina*); por su parte, Liu *et al*. (2010) menciona que el silicato de sodio ocasionó un daño a la membrana plasmática de esporas de *Penicillum. digitatum* e inhibió la germinación de esporas, la elongación del tubo germinativo y el crecimiento micelial en condiciones *in vitro*, además de que redujo significativamente, en ensayos *in vivo* la podredumbre verde en frutos cítricos. Asimismo, el silicato de sodio tuvo una concentración mínima inhibitoria del 0.25% para *P. digitatum* y *P. italicum* en condiciones *in vitro*, y además, tratamientos poscosecha con esta misma sustancia, redujeron significativamente las podredumbres verde y azul en frutos cítricos (Youssef *et al*., 2012).

2.2.4. Mecanismo de acción del silicio

El silicio puede tener un efecto directo sobre el patógeno dañando la membrana celular del hongo (Liu *et al*., 2010). El papel de las paredes celulares silicificadas en la protección de las plantas contra los patógenos no puede descartarse completamente, otros resultados sugieren que el Si actúa en la activación y/o regulación de mecanismos de defensa naturales de las plantas (Chérif *et al*., 1992; Samuels *et al*., 1991). Aunque algunos de estos mecanismos específicos aún no son bien conocidos, sí se han reportado otros, como la acumulación de lignina o la síntesis de compuestos fenólicos y enzimas relacionadas con la patogénesis en plantas infectadas tratadas con Si (Chérif *et al*., 1992; Epstein, 1999).

Datnoff *et al*. (2007) y Brunings *et al.* (2009) mencionaron que los mecanismos bioquímicos y fisiológicos potenciados por el Si incluyen una mayor concentración de compuestos fenólicos, lignina y fitoalexinas, un aumento en las actividades de las enzimas de defensa como las quitinasas y las β-1,3-glucanasas y la rápida y fuerte transcripción de genes relacionados con la resistencia del huésped. Ma y Yamaji (2008), menciona que el Si se deposita debajo de la cutícula y forma una doble barrera con esta, lo que mecánicamente impide la penetración del tubo germinativo y por, lo tanto detiene el proceso de infección.

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CAPÍTULO III. FIRST REPORT OF PAPAYA FRUIT ANTHRACNOSE CAUSED BY *Colletotrichum brevisporum* IN MÉXICO

Submitted to la Revista Mexicana de Fitopatología

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Anthracnose is the most important postharvest disease on papaya fruit worldwide with postharvest losses ranging from 25 to 40% (Singh, 2010). In June 2019, papaya fruit (*Carica papaya* L. cv. Maradol) showing post-harvest anthracnose symptoms were obtained from the local market in Cárdenas, Tabasco, México. Fruit affected by anthracnose showed sunken, prominent, dark brown to black lesions. Small pieces (5-7 mm) of diseased tissue (composed of ~20% diseased tissue and ~80% healthy tissue) were excised of the lesion edge, disinfected for 2 min in an aqueous solution of 1.0% NaOCl, rinsed twice with sterile distilled water, and collocated into plate containing potato dextrose agar (PDA). A mycelial agar disc of 5 mm in diameter was transferred to a fresh PDA plate in order to obtain a pure culture. Macroscopic colony and microscopic characteristics of one isolate were observed after mycelial growth for

6 d at 25°C. Young colony showed color dark green with white margin (Figure 3.1), and developed colony showed conidial mass of color orange (Figure 3.2).

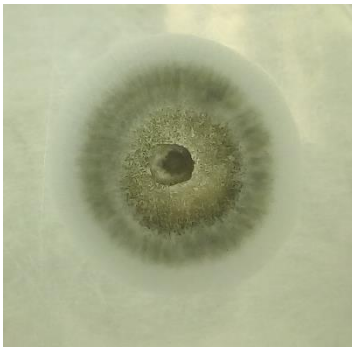

Figure 3.1. Colony of *C. brevisporum* growing on PDA, incubated at 25°C for 3 days.

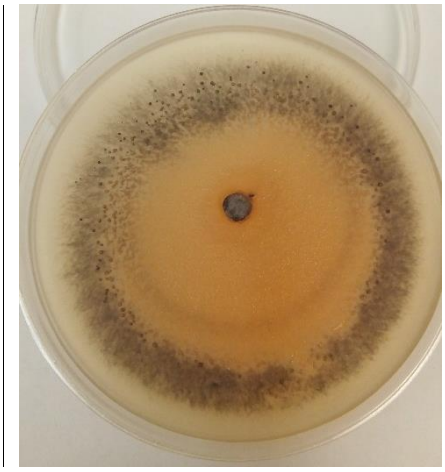

Figure 3.2. Colony of *C. brevisporum* on PDA medium showing conidial mass in orange color, incubated at 25 °C for 9 days.

Conidia were hyaline, cylindrical with round ends, smooth-walled, aseptate, guttulate, and 15.0 (12.3 to 17.2) µm X 5.5 (4.7 to 6.1) µm (n= 40) (Figure 3.3), typical of *Colletotrichum* spp (Weir *et al*., 2012). DNA extracting, amplifying, sequencing (ITS 1: 5´-

TCCGTAGGTGAACCTGCGG-3´; ITS 4: 5´-TCCTCCGCTTATTGATATGC-3´), and the internal transcribed spacer (ITS1-18S-ITS4-28S rRNA) were carried out to accurately identify the species. Sequence of the papaya isolate was 99.82 similar to those of *Colletotrichum brevisporum* (GenBank Accession No. MT232983).

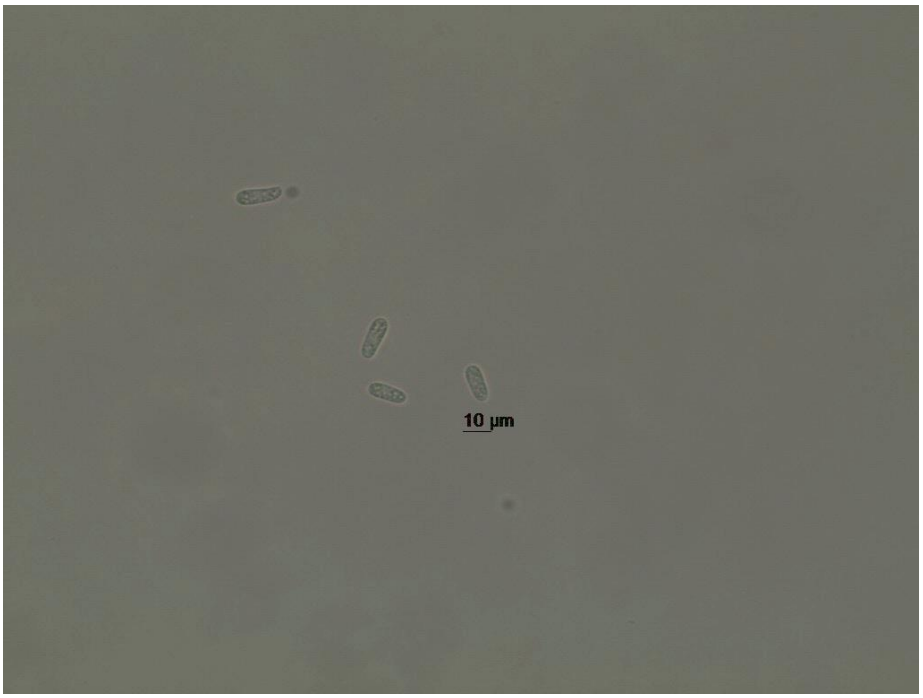

Figure 3.3. Conidia of *C. brevisporum*

In this study, conidia of the papaya isolate were bigger than those described for *C. brevisporum* 13.5 (10.5 to 17.1) µm X 3.8 (2.1 to 4.8) µm, respectively) (Vieira *et al*., 2013), which may be due to variations in incubation temperatures or to typical variation in conidial size in *Colletotrichum* species. For the pathogenicity testing an experiment was conducted with papaya cv. Maradol using a completely randomized design. The experiment consisted of two treatments: A) inoculated wound, and 2) non-inoculated wound. Each treatment was composed by four replicates with four wounds each. Wounds of a 2 mm diameter and 1-2 mm

depth were done with the tip of a sterile wooden stick on the fruit equatorial region. The inoculation consisted in collocate 30 µl of a conidial suspension of 10^6 conidia ml^{-1} of *C. brevisporum*. The inoculated fruit were incubated at 24 ± 1°C and 85-90% RH for 6 d. After 6 d, typical symptoms from anthracnose were observed on papaya fruit (Figure 3.4). The fungus *C. brevisporum* was successfully re-isolated from symptomatic fruit to fulfill Koch's postulates.

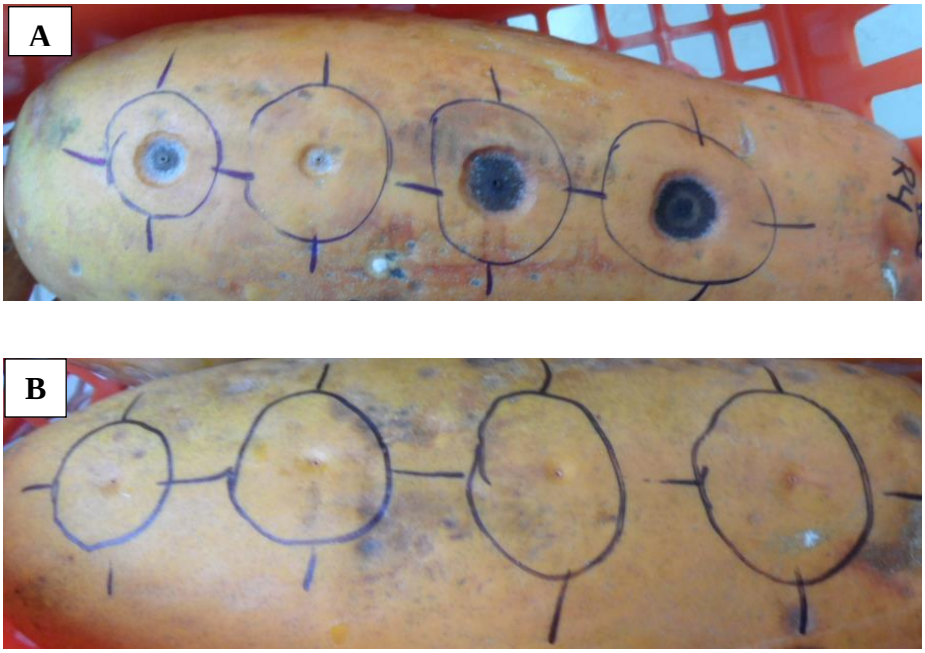

Figure 3.4. Anthracnose symptoms on papaya fruit cv. Maradol after 6 d of incubation at 24 °C. A**.** Inoculated wound, B. Non-inoculated wound

C. brevisporum has been reported to cause anthracnose on papaya fruit, first in Brazil (Viera *et al*., 2013), then in Australia (Shivas *et al*., 2016) and recently in Taiwan (Duan *et al*., 2018). To our knowledge this is the first report of *C. brevisporum* on papaya fruit in México.

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CAPÍTULO IV. ANTIFUNGAL EFFECT OF SILICON AGAINST ANTHRACNOSE ON FRUIT IN PAPAYA MARADOL

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Abstract

The curative and preventive antifungal activity of postharvest Silicon (Si) treatments against papaya anthracnose was determined on papaya cv. Maradol artificially inoculated with *C. brevisporum* and incubated at 25-26 °C and 80-90 % RH for 5 or 6 d. *In vitro* effective concentration range of potassium silicate (PS) and sodium silicate (SS) was taken into account as reference for subsequent *in vivo* primary experiments. Effective concentrations and type of treatment (curative or preventive) were selected in *in vivo* primary screenings for subsequent experiments. SP and SS at 1.5 and 1.0 %, respectively, were tested for 30, 60, 90 or 180 s to determine the best dip treatment time in preventive treatments. Dips of 1.5 % PS and 1.0 % SS

at 20 °C for 60 and 90 s were selected and applied alone or in combination with 250 μL L^{-1} of the conventional fungicide thiabendazole. The concentrations of 3.0 and 2.5 % PS were the most effective to inhibit the mycelial growth of *C. brevisporum* (93 and 91 %, respectively), and SS at these same concentrations inhibited totally the mycelial growth of the fungus. In *in vivo* primary screenings, although no significant curative effect of silicon was observed on postharvest anthracnose disease, PS at 1.5 % and SS at 1.0 % consistently reduced the anthracnose incidence until 6.3 and 0.0 %, respectively. All the treatments with SS consistently reduced the anthracnose severity until a range of 0.0-1.4 mm of lesion diameter. Anthracnose severity on papaya fruit were not reduced by treatment with PS for 6 d of incubation. The best dip times for PS at 1.5 % and SS at 1.0 % were 60 and 90 s, respectively. These treatments of dips reduced the anthracnose incidence until 6.7 and 0.0 % after 5 d of incubation. PS at 1.5 % for 60 s and SS at 1.0 % for 90 s, at 20 °C, combined with low doses of TBZ did not improved the control of anthracnose disease. These results showed that Si aqueous solutions, applied at room temperature, might be an interesting nonpolluting control alternative to be included in papaya postharvest disease control programs in the future.

Key words: *Colletotrichum brevisporum*, papaya, low-toxicity substances, alternative disease control, Thiabendazole

4.1. Introduction

Anthracnose of papaya caused by *Colletotrichum* species is the more important postharvest disease in the worldwide. The contagion can initiate when flowering starts then the pathogen remains latent until the fruit begins its development, after harvest, during the fruit storage and shelf-life period (de Capdeville *et al*., 2007; Lima Oliveira *et al*., 2018). The postharvest papaya anthracnose, is observed on the fruit which severely affects the marketability of these (Ademe *et al*., 2014).

The control of the anthracnose is based on mainly in the use of synthetic chemical fungicides such as benomyl, thiabendazole and prochloraz (Zavala-León *et al*., 2005). However, the use of these chemical fungicides can accumulate residues in the papaya fruit, contaminate to the environment and induce resistance of the fungus to these fungicides. Furthermore, it is important to research alternatives to conventional synthetic chemical control more friendly with the environment such as the silicon.

Silicon has shown its potential for anthracnose control in different fruit species. For instance, Zainuri *et al*. (2005) demonstrated the potential of soluble silicon in inducing resistance of mango fruits against anthracnose (*Colletotrichum gloeosporioides*). Moreover, Bi *et al*. (2006) evaluate postharvest dip treatments with silicon on the development of rot caused by *Alternaria alternate*, *Fusarium semitectum* and *Trichothecium roseum* on melon fruit of the New Queen and 8601 varieties, finding that the silicon to concentration of 100 mM reduced up to 67 and 72 % for *T. roseum* in the New Queen and 8601 varieties, respectively. Likewise, Bekker *et al*. (2009) found that potassium silicate completely suppressed the mycelial growth of *C. gloeosporioides* at a concentration of 20 mL L^{-1}. In another study, Liu *et al*. (2010) reported that sodium silicate damaged the plasma spore membrane of *Penicillium digitatum*

and inhibited spore germination, germ tube elongation, and mycelial growth under *in vitro* conditions. Likewise, sodium silicate at 0.5 % reduced until 45 % of incidence of green mold (*Penicillum digitatum*) in citrus fruits in preventive treatments through *in vivo* primary experiments. Moscoso-Ramírez and Palou (2014) revealed that 90 mM potassium silicate applied in curative and preventive treatment, significantly reduced the incidence of blue mold (*Penicillum italicum*) by 40 and 52 %, respectively in citrus fruits *in vivo* primary screenings. These same researchers reported that 90 mM potassium silicate applied in dips at 20 °C and an immersion time of 60 s, reduced the incidence and severity of green mold to 37 and 50 %, respectively, in 'Lanelate' oranges stored at 20 °C for 7 days. Likewise, Ge *et al*. (2017) confirmed that at different concentrations of sodium silicate there was a significant inhibition of mycelial growth and spore germination of *Trichothecium roseum*. In another more recent study, Elsherbiny and Taher (2018), reported that 10 mM de sodium metasilicate inhibited the micelial growth of *Sclerotia sclerotiorium*, with a mycelial growth inhibition of 92.2 % after 6 d of incubation.

On the other hand, several studies have been reported using other generally recognized as safe (GRAS) substances to control postharvest papaya anthracnose such as Chitosan (Lima-Oliveira *et al*., 2018; Vilaplana *et al*., 2020), and calcium chloride (Ayón-Reyna *et al*., 2017; dos Passos Braga *et al*., 2019).

This study is focused to research strategies of a non-polluting control alternative to conventional chemical control, which will help to contributing at acknowledge of anthracnose disease in papaya fruits caused by the *Colletotrichum brevisporum* fungus through the use of chemicals that are more friendly to the environment and human health such as silicon (Si) in its forms of potassium silicate (PS) and sodium silicate (SS). Furthermore, to our knowledge,

there are no research reports on the antifungal activity of Si in anthracnose, specifically on the papaya fruit. In addition, PS was included in 2010 in the list of substances accepted in organic agriculture in USA (USDA AMS, 2010). Based on the above-mentioned, the objectives of the present research work were to: 1) Determine the *in vitro* effect of silicon on micelial growth of *Colletotrichum brevisporum*, 2) Evaluate the *in vivo* effect of silicon on anthracnose through *in vivo* primary screenings experiments, 3) Determine the effect of dip time in the silicon effectiveness on the anthracnose, and 4) Evaluate the silicon effect alone or combined with low doses of thiabendazole.

4.2. Materials and methods

4.2.1 *In vitro* effect of the silicon on mycelial growth of *Colletotrichum brevisporum*

In vitro experiments were conducted to determine the effect of silicon on its forms of potassium silicate (SP) and sodium silicate (SS) on the mycelial growth of *Colletotrichum brevisporum.*

Potassium and sodium silicate were tested in the concentrations following: 0.30, 0.60, 0.80, 1.0, 1.5, 2.0, 2.5 and 3.0 % plus the control. A completely randomized experimental design was performed. Each treatment consisted of 5 replicates (each Petri dish was a replicate).

A stock aqueous solution was prepared at the concentration of 20 % silicate (potassium silicate for the first experiment, and sodium silicate for the second experiment), which was used to prepare the desired concentrations of silicate (treatments) and then added to the medium of potato dextrose agar (PDA), when it was at a temperature of 45-50 °C.

Approximately 20 mL of PDA medium with the desired SP or SS concentrations was poured into each Petri dish.

24 h after emptying into the Petri dishes, a 5 mm mycelial disk with PDA medium from a 10-15 d old *C. brevisporum* colony was taken and placed in the center of another Petri dish (100 mm diameter) with fresh PDA, containing the desired concentrations (Treatments) of silicate (potassium silicate for the first experiment, and sodium silicate for the second experiment). These experiments were repeated twice.

In both experiments, the radial mycelial growth of *C. brevisporum* was measured, for which two diameters (mm) of the pathogen colony were measured perpendicular to each other, after 9 d of incubation of *C. brevisporum* at 25 °C and the variable was expressed as the inhibition of mycelial growth in percentage (% ICM), which was calculated using the following formula:

$$\% \text{ ICM} = \text{DC-DT/DC} * 100$$

Where:

DC = Diameter of the colony in the control in mm.

DT = Diameter of the colony in the treatment in mm.

4.2.2 *In vivo* effect of silicon on the anthracnose disease

4.2.2.1 Fruit

The experiments were conducted with papaya fruit cv. Maradol at ripening stage 4, bought from a seller in Ranchería Corregidora, Centro, Tabasco (17.956146,-93.124239). The fruit were free of damages with a uniform size. The fruit were washed with clear water and

disinfected with an aqueous solution of 3 % NaOCl by immersion for two min, then rinsed with water to eliminate the residual chlorine and dried at room temperature.

4.2.2.2 Preparation of fungal inoculum

The conidia of the pathogen were taken from the surface of a 10-15 d old *Colletotrichum brevisporum* colony from the Petri dishes with the help of a sterile loop and a suspension was prepared in sterile water with 0.05 % ($w.v^{-1}$) of Tween 80 of 10^6 conidia mL^{-1} using a haemocytometer to adjust the concentration.

4.2.2.3 Inoculation of *Colletotrichum brevisporum*

To carry out the inoculation of the fungus, equidistant wounds 1 mm in diameter and 2 mm deep were previously made with a sterile wooden toothpick on the equatorial region of the fruit. 30 µL of the conidia suspension of *C. brevisporum* were placed in each wound site with the help of a micropipette.

4.2.2.4 *In vivo* Primary screenings

Two *in vivo* primary screenings were performed, the first experiment with potassium silicate (39.2 % a.i., Ag Sil® 21 PQ Corporation) and the second experiment with sodium silicate (38.7 % a.i., sodium silicate, 1.4 g cm^3 PQ Corporation) to determine the most effective concentration for the curative and preventive effect of silicon on anthracnose on papayas cv. Maradol. Both silicates were tested at the concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %

plus the control. The selection of the tested concentrations range was based on the results obtained in the *in vitro* experiments.

For both experiments, the preparation of a conidial suspension of *Colletotrichum brevisporum* was carried out as described in subsection 4.2.2.2. Preparation of fungal inoculum. The inoculation was performed following the procedure in section 4.2.2.3. Inoculation of *Colletotrichum brevisporum*.

A stock aqueous solution was prepared at the concentration of 20 % silicate (potassium silicate for the first experiment, and sodium silicate for the second experiment), from this the desired concentrations were made by the dilution method.

Treatments were performed, placing an aliquot of 30 µL of aqueous silicate solution using a micropipette at the same site where the fungus was inoculated about 1-2 h before (curative treatment) or after (preventive treatment). The control fruit was treated with 30 µL of sterile distilled water.

For both experiments, four replicates (four wounds per replicate, using one papaya fruit with four wounds each fruit) were used per each treatment (four papaya fruits).

The inoculated and treated fruit was incubated at 25-26 ºC and 80-90 % RH for 6 d. The incidence (% of infected wounds) and the severity (lesion diameter in mm) of anthracnose disease, were assessed 6 d after inoculation. Treatments were arranged in a completely randomized experimental design.

4.2.2.5 Time of dip treatments

Small-scale trials were conducted with papaya fruit cv. Maradol to stablish the best dip time. The inoculation of *C. brevisporum* was carried out 1-2 h after the treatment applications (preventive treatment) following the procedure mentioned in the inoculation of *C. brevisporum* section. Plastic container containing 18 L of an aqueous solution of 1.0 % PS (10 g L^{-1}) or 1.5 % SS (15 g L^{-1}) were used. These concentrations and the treatment type were selected according to previous results obtained in *in vivo* primary screenings. Papaya fruit were collocated into multi-perforated wall plastic containers of 10 L, exactly fitting in the above-mentioned containers, and completely immersed in the treatment solution for 30, 60, 90 and 180 s. Control fruit were dipped in water alone at room temperature. Four replicates were used per treatment, and each replicate consisted of 8 wounds made on the equatorial region of one fruit (4 wounds on one side and the others 4 wounds on the opposite side). Treated fruit were incubated at 25-26 °C and 80-90 % RH for 5 d, into an incubation room located in the Laboratorio de control Biológico del Campus Tabasco. Disease incidence (Percentage of infected wounds) and severity (lesion diameter in mm) were assessed after 5 d of incubation.

4.2.2.6 Effect of the silicon alone or combined with low doses of thiabendazole

To determine the effect of the combination of silicon with the tiabendazol fungicide (TBZ; 2-(4-Tiazolil)-1H-benzimidazol; MERTECT®340 465.3 g of a.i. L^{-1}; Arysta LifeScience, ciudad de México, México) to control anthracnose, two experiments were conducted, one with potassium silicate and another one with sodium silicate. In the first experiment, the following treatments were applied: (1) water alone (control), (2) non-inoculated control (3) 1.5 % PS (1.5 % PS), (4) thiabendazole at 250 µL L^{-1} (250 TBZ), (5) thiabendazole at 500 µL L^{-1} (500

TBZ), (6) combination of 1.5 % PS with 250 µL L^{-1} TBZ (PS + TBZ), and (7) quarantine treatment (QT; 48 °C for 20 min). In the second experiment, the following treatments were evaluated: (1) water alone (control), (2) non-inoculated control (3) 1.0 % SS (1.0 % SS), (4) thiabendazole at 250 µL L^{-1} (250 TBZ), (5) thiabendazole at 500 µL L^{-1} (500 TBZ), (6) combination of 1.5 % SS with 250 µL L^{-1} TBZ (SS + 250 TBZ), and (7) quarantine treatment (QT; dips for 20 min at 48 °C). The combined treatments of each silicate with TBZ were prepared mixing the desired dose in an aqueous solution of 20 L contained into a plastic container and manually stirred with a clear plastic pod. TBZ was used at two doses: one commercial dose and another lower than commercial dose. Fungal inoculation and dip treatments were performed following the above-mentioned procedure. Dip conditions were room temperature and immersion time of 60 s for treatments with PS alone and combined (PS + TBZ) and 1.0 % SS alone and SS + TBZ treatments, with dip time of 90 s. Each treatment was applied to four replicates of eight wounds each (for each replicate was used one papaya fruit). Disease incidence and severity were assessed after 5 d of incubation at 25-26 °C and 80-90 % RH.

4.3. Statistical analysis

The data of incidence (percentage) and severity (mm) of anthracnose were analyzed by analysis of variance (ANOVA) with Statgraphics Plus software (v 5.1). In the case of the incidence in percentage of anthracnose, the data were transformed to the arcsine of the square root of the proportion of infected wounds to ensure homogeneity of variances. Statistical significance was considered at the $p = 0.05$ level. Fisher's least significant difference test (LSD) was applied to separate means. Values shown are untransformed means.

4.4. Results

4.4.1 Effect of the silicon on mycelial growth of *Colletotrichum brevisporum*

The concentrations of 3.0 and 2.5 PS, were the more effective to reduce the radial mycelial growth of *C. brevisporum*, with values of 93 and 91 % of inhibition of mycelial growth, respectively ($P < 0.05$), with respect to the control plate after 9 d of incubation at 25 °C, followed by the concentrations of 2.0 and 1.5 % PS, with values of 77 and 79 %, respectively. The concentration of 1.0 % PS modestly reduced the radial mycelial growth of *C. brevisporum*, with a value of 26 % of inhibition of mycelial growth; while the rest of the concentrations (0.3, 0.6 and 0.8 %) evaluated of PS did not significantly reduced the radial mycelial growth of *C. brevisporum*, ranging from 0 to 2 % of inhibition of mycelial growth (Fig. 4.1).

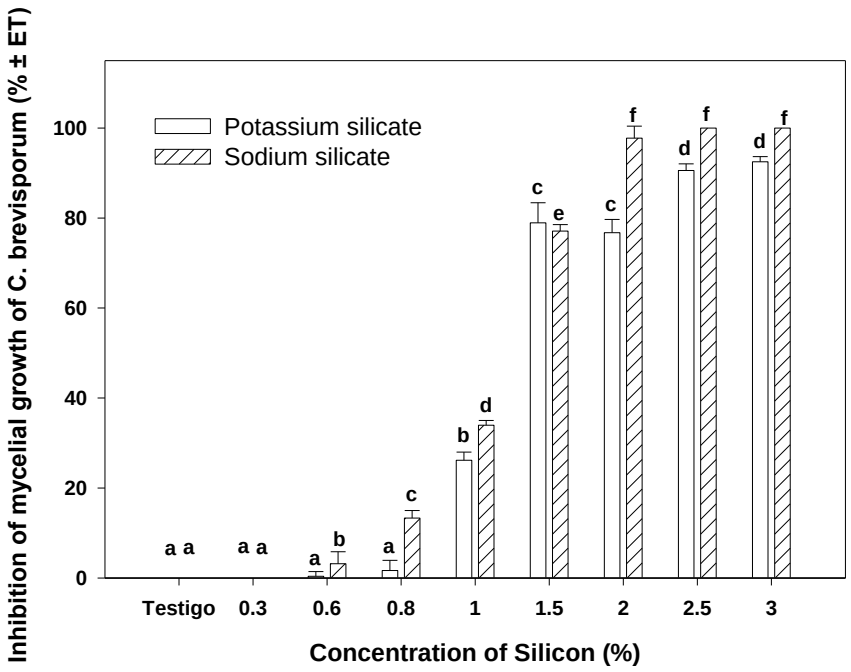

Figure 4.1. Effect of silicon on the micelial growth of *C. brevisporum* at 25°C after 9 d of incubation.

Regarding to the effect of silicon in its form of sodium silicate, the concentrations of 3.0 and 2.5 % completely inhibited the radial mycelial growth (100 % of inhibition of mycelial growth) of *C. brevisporum* compared with the control plate (0 % of inhibition of mycelial), after 9 d of incubation at 25 °C. Likewise the concentration of 2.0 % SS was highly effective to reduce the mycelial growth of *C. brevisporum* with a value of 98.0 % of inhibition of mycelial growth, followed by the concentrations of 1.5, 1.0, 0.8 and 0.6 % SS, with values of 77.0, 34.0, 13.0 and 3.0 % of inhibition of mycelial growth, respectively (Fig. 4.1).

4.4.2 *In vivo* primary screenings

4.4.2.1 Curative effect of silicon on anthracnose disease

In general, no significant curative effect of silicon in its form of potassium silicate was observed on the incidence and severity of anthracnose disease on fruit of papaya cv. Maradol, inoculated artificially 1-2 h before application of treatments with potassium silicate with *C. brevisporum* and incubated at 25-26 °C and 80-90 % RH for 6 d. The incidence of anthracnose disease ranged from 64.3 to 81.3 % by application of the different concentrations of PS (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % of PS) with respect to the control treatment (93.8 % of incidence). Likewise, the severity (lesion diameter) of anthracnose ranged from 4.9 to 10.4 mm of lesion diameter when applied the different concentrations of PS (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %; Fig. 4.2).

Regarding the effect of the silicon in its form of sodium silicate (SS) on postharvest anthracnose, no significant curative effect was found on the incidence and severity of anthracnose decay on papaya cv. Maradol, inoculated artificially 1-2 h before application of treatments with SS, with *C. brevisporum* and incubated at 25-26 °C and 80-90 % RH for 6 d.

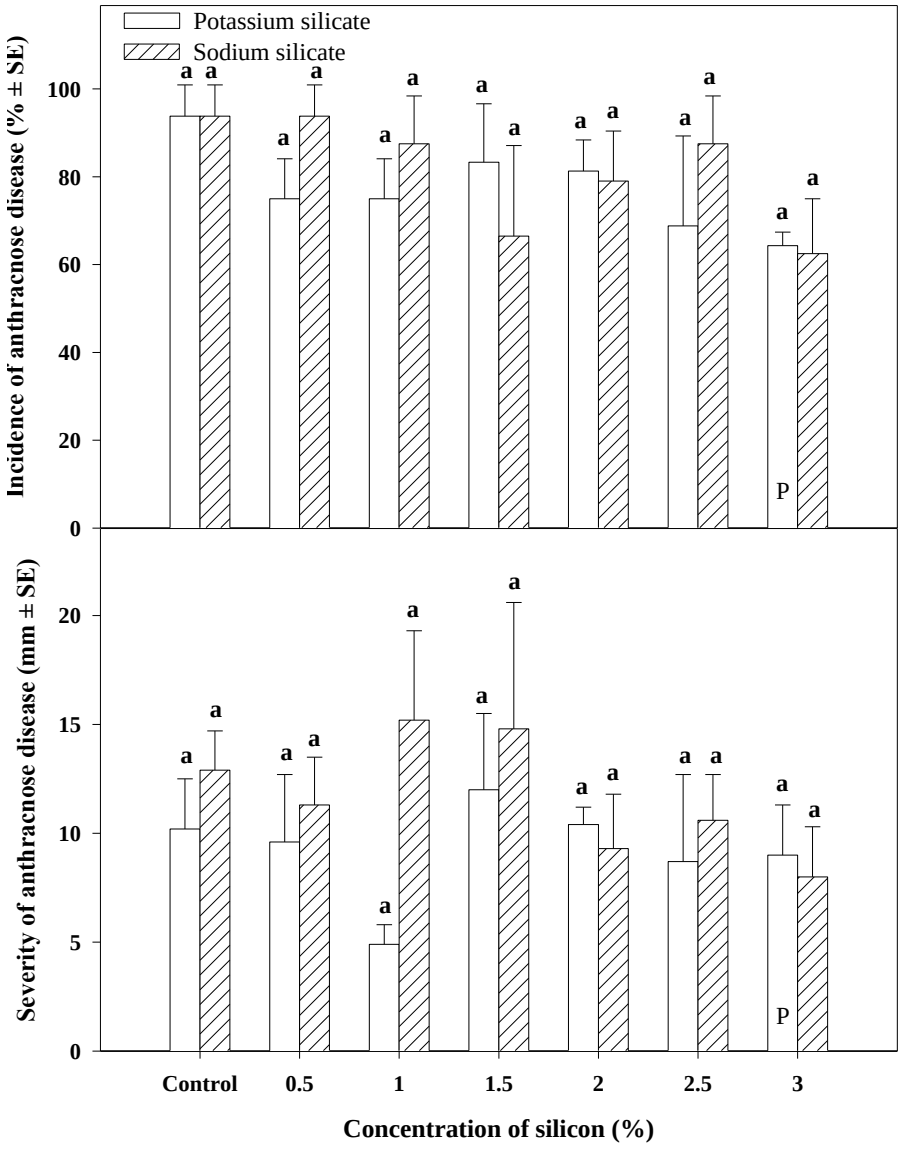

Figure 4.2. Curative effect of silicon at different concentrations on anthracnose disease in *in vivo* primary screenings with papaya cv. Maradol artificially inoculated with *C. brevisporum*, treated 1-2 h later, and incubated for 6 d at 25-26°C and 80-90% RH. "P" indicates Phytotoxicity on the fruit rind.

The incidence of anthracnose disease ranged from 62.5 to 93.8 %, in the different tested concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % of SS) in comparison with the control treatment (93.8 % of incidence). Moreover, the severity varied from 9.3 to 15.2 mm of lesion diameter in the different treatments with SS when compared with the control treatment (12.9 mm of lesion diameter; Fig. 4.2).

4.4.2.2 Preventive effect of silicon on anthracnose disease

In overall, only the treatment with 1.5 % silicon in its form of potassium silicate was significantly effective to reduce the incidence (6.3 % of incidence) of anthracnose disease on fruit of papaya cv. Maradol, inoculated artificially 1-2 h after application of treatments with potassium silicate with *C. brevisporum* and incubated at 25-26 °C and 80-90 % RH for 6 d; while the incidence on the control treatment was of 87.5 %. The concentrations of 3.0 and 2.5 % silicon considerably reduced the incidence of anthracnose; but they had no significant differences with the control treatment (Fig. 4.3). The rest of the concentrations of PS (0.5, 1.0, and 2.0 % of PS) did not reduce anthracnose incidence, with an incidence ranging from 81.3 to 100.0 % (Fig. 4.3). In contrast, the severity (lesion diameter) of anthracnose was not significantly reduced by silicon in its form of potassium silicate in the different concentrations tested (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % of PS) with respect to the control treatment, and it ranged from 2.8 to 10.2 mm of lesion diameter; while anthracnose severity in the control treatment was of 11.9 mm of lesion diameter.

With regards at effect of the silicon in its form of sodium silicate (SS) on postharvest anthracnose incidence, all treatments with silicon in its form of SS consistently controlled the incidence of anthracnose decay. The anthracnose incidence was significantly reduced passing

from 93.8 % on the control treatment to 0.0, 6.3, 6.3, 6.3, 12.5 and 12.5 % in the 1.0, 0.5, 2.0, 2.5, 1.5 and 3.0 % treatments of SS, respectively.

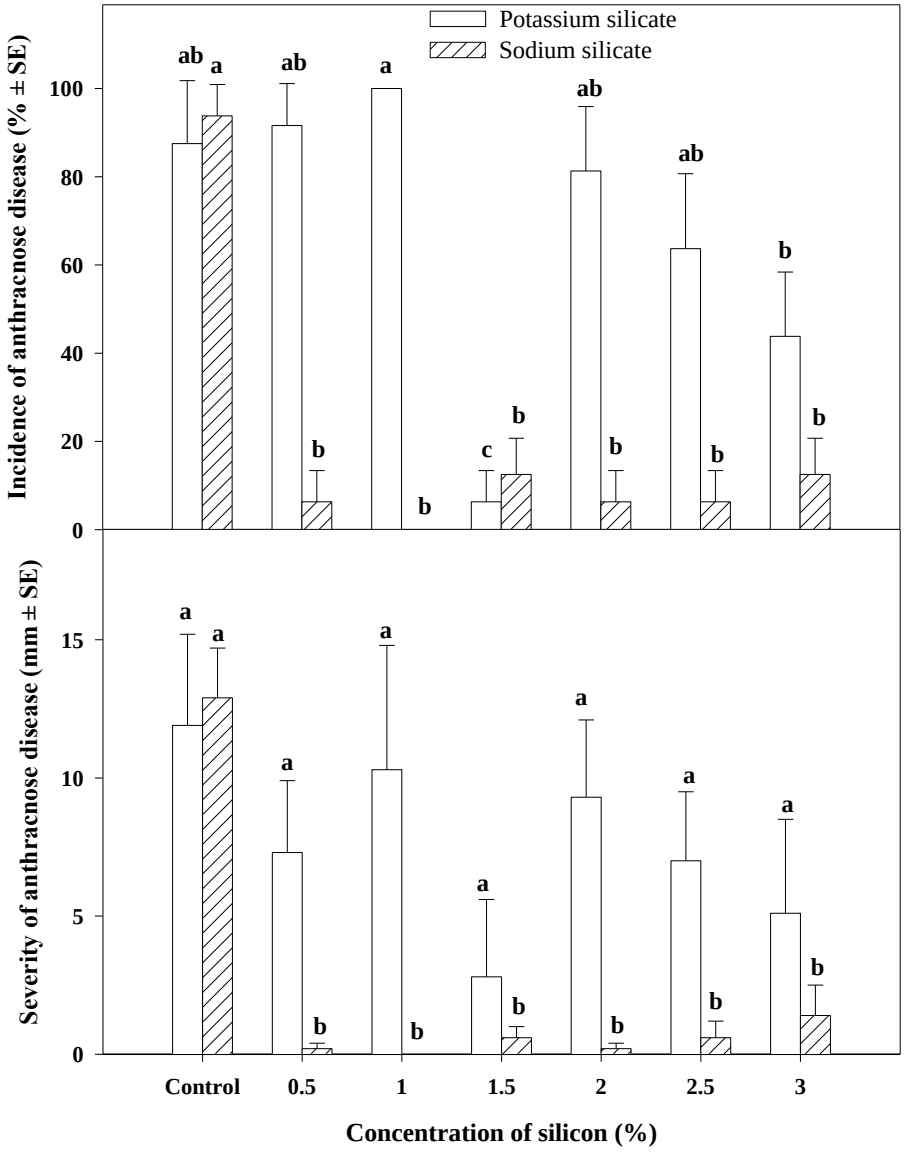

Figure 4.3. Preventive effect of silicon at different concentrations on anthracnose disease in *in vivo* primary screenings with papaya cv. Maradol artificially inoculated with *C. brevisporum*, treated 1-2 h before, and incubated for 6 d at 25-26°C and 80-90% RH.

Similarly, the severity of anthracnose decay was effectively reduced by all the treatments with SS. The lesion diameter passed from 12.9 mm on the control treatment to 0.0, 0.2, 0.2, 0.6, 0.6 and 1.4 mm in the 1.0, 0.5, 2.0, 1.5, 2.5 and 3.0 % treatments of SS, respectively (Fig. 4.3).

4.4.3 Effect of the time of preventive dip treatments

According to the results obtained in the in vivo primary screenings, PS at 1.5 % and SS at 1.0 % applied in preventive treatment were chosen as the best treatments to be tested in preventive postharvest dips in small-scale trials.

In general, dips with PS at 1.5 % applied at 20 °C for 30, 60, 90 or 180 s significantly reduced the incidence of anthracnose disease with respect to the control treatment (84.5 % of incidence of anthracnose) on papayas cv. Maradol treated, inoculated 1-2 h later, and incubated for 5 d at 25-26 °C and 80-90 % RH.

The anthracnose incidence were 15.0, 6.7, 28.6, or 8.3 %, respectively (Fig. 4.4), but had no significant differences among them. However, since a practical point of view the dip time was selected based on anthracnose incidence. Thus, the dip time of 60 s was chosen as the best treatment to be used in subsequent experiments. Similarly, dips with SS at 1.0 % at 20 °C for 30, 60, 90 or 180 s significantly reduced the incidence of anthracnose disease with respect to the control treatment, with anthracnose incidence values of 9.4, 3.1, 0, 3.1, or 84.5 %, respectively (Fig. 4.4).

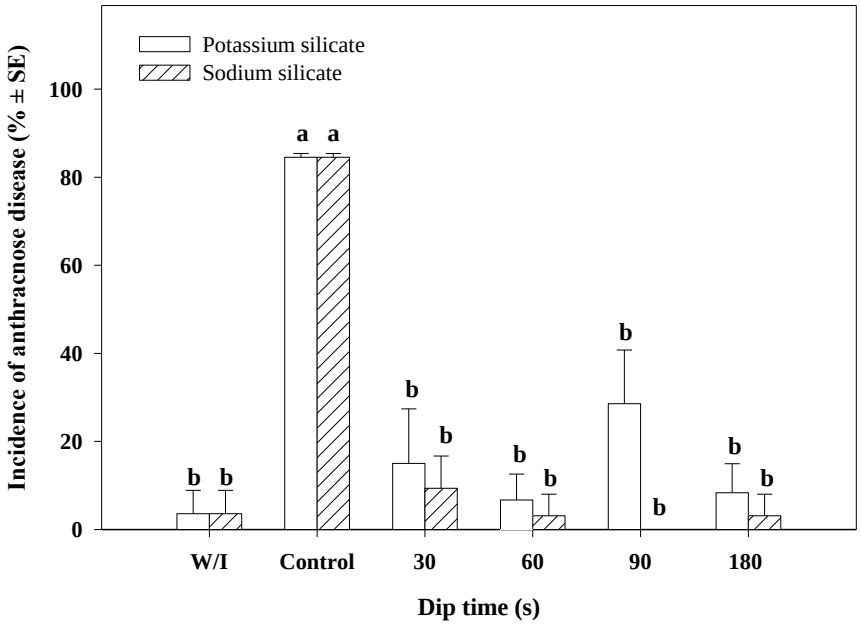

Figure 4.4. Effect of dip time on the effectiveness of 1.5% potassium silicate (PS) and 1.0% sodium silicate (SS) to control the incidence of anthracnose disease on papayas cv. Maradol artificially inoculated with *C. brevisporum*, treated 1-2 h before, and incubated for 5 d at 25-26°C and 80-90% RH.

Irrespective of control treatment, the dip times had no significant differences from each other, but following the same criteria above-mentioned to select a dip time, the dip time of 90 s was selected as the best treatment to be used in further assayed.

4.4.4 Effect of the silicon alone or combined with TBZ

Dips with 1.5 % PS applied alone at 20 °C for 60 s on papaya fruit cv. Maradol in preventive treatment was the most effective to reduce the incidence of anthracnose disease in comparison with the rest of the treatments (Fig. 4.5), with the exception of the quarantine treatment after 5

d of incubation at 25-26 °C and 80-90 % RH. The incidence of anthracnose disease when applied PS alone was 32.6 % while in the 500 TBZ, PS + TBZ, 250 TBZ, and control treatments the papaya anthracnose incidence was 46.9, 50.0, 73.8, and 70.0 %; respectively. The quarantine treatment (48 °C for 20 min) was consistently the most effective to reduce the anthracnose incidence, with an incidence of 13.4 %. A similar trend was observed for the anthracnose severity, PS (1.5 %) applied alone in dips was the most effective to reduce the disease severity compared with rest of the treatments (Fig. 4.5), with the exception of the quarantine treatment. Lesion diameter of 2.8 mm were observed by application of PS, while in the PS + TBZ, 500 TBZ, 250 TBZ, and control treatments the papaya anthracnose incidence was 5.1, 5.3, 6.4, and 9.2 mm; respectively. Likewise, the quarantine treatment was the most effective to reduce anthracnose severity, with a value of 1.6 mm of lesion diameter.

The combination of 1.0 % SS plus 250 TBZ applied in dips at 20 °C for 90 s on papaya fruit cv. Maradol in preventive treatment did not significantly improve the control of the anthracnose incidence in comparison with the 1.0 % SS treatment applied alone (Fig. 4.6), but both treatments were superior to control the anthracnose incidence than those of the 500 TBZ treatment after 5 d of incubation at 25-26 °C and 80-90 % RH.

The incidence of anthracnose disease by application of PS alone or in combination with 250 TBZ was 25.0 and 34.4 %, respectively while in the 500 TBZ treatment was of 46.9 % of anthracnose incidence. Moreover, the combination of SS (1.0 %) with 250 TBZ did not significantly reduce the anthracnose severity compared with that of the 1.0 % SS treatment applied alone, with values of 4.2 and 3.6 mm of lesion diameter, respectively (Fig. 4.6); however, the effectivity on the anthracnose severity of both treatments were similar of that obtained by the 500 TBZ treatment (5.3 mm of lesion diameter).

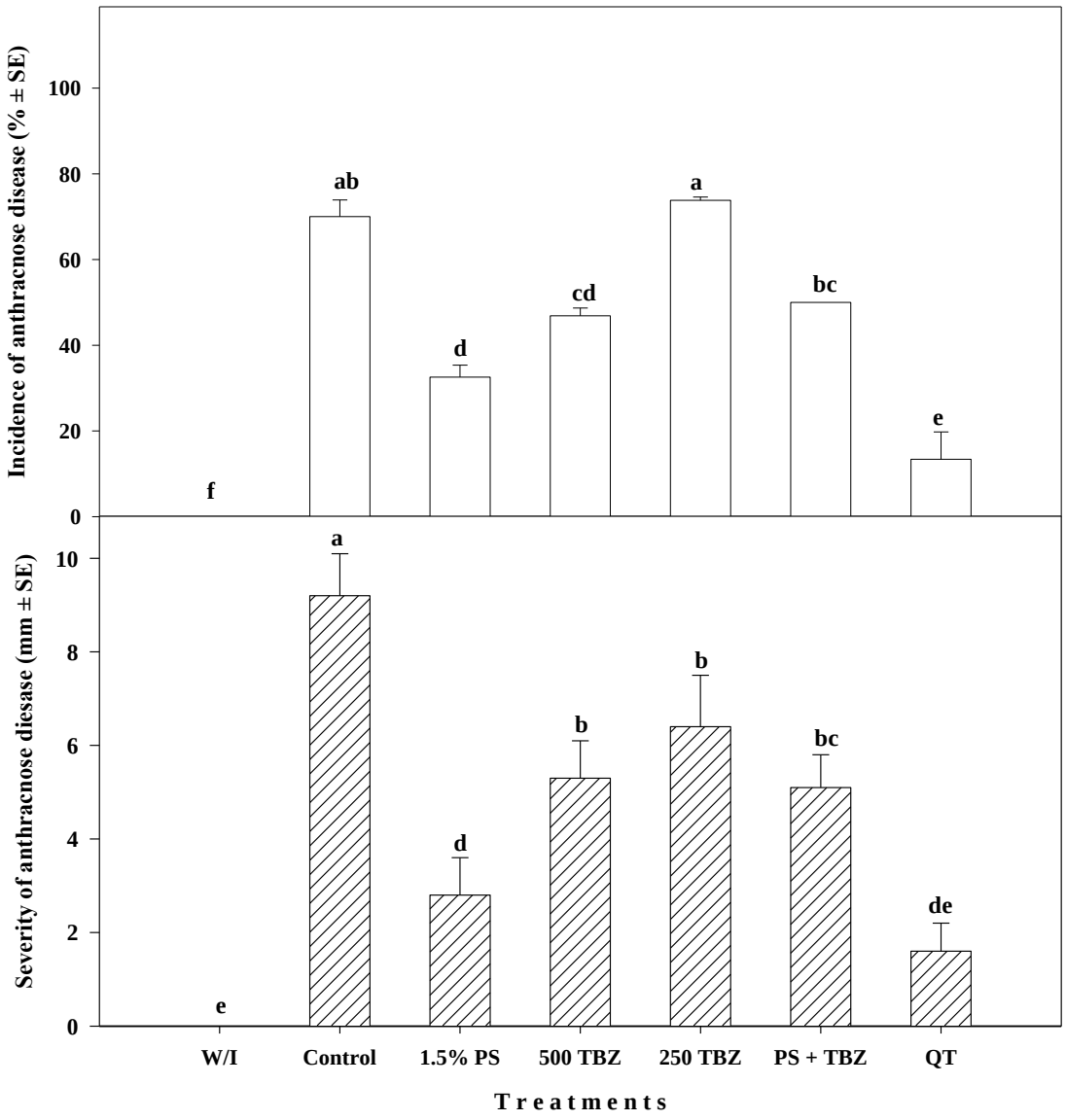

Figure 4.5. Effectiveness of 1.5% potassium silicate alone (PS), 250 ppm fungicide thiabendazole (250 TBZ), 500 ppm thiabendazole (500 TBZ), combination of 1.5 % PS and 250 ppm TBZ (PS + TBZ) and quarantine treatment (48°C for 20 min; QT) to control anthracnose disease on papayas cv. Maradol artificially inoculated with *C. brevisporum*, treated 1-2 h before for 60 s at 20°C, and incubated for 5 d at 25-26°C and 80-90% RH.

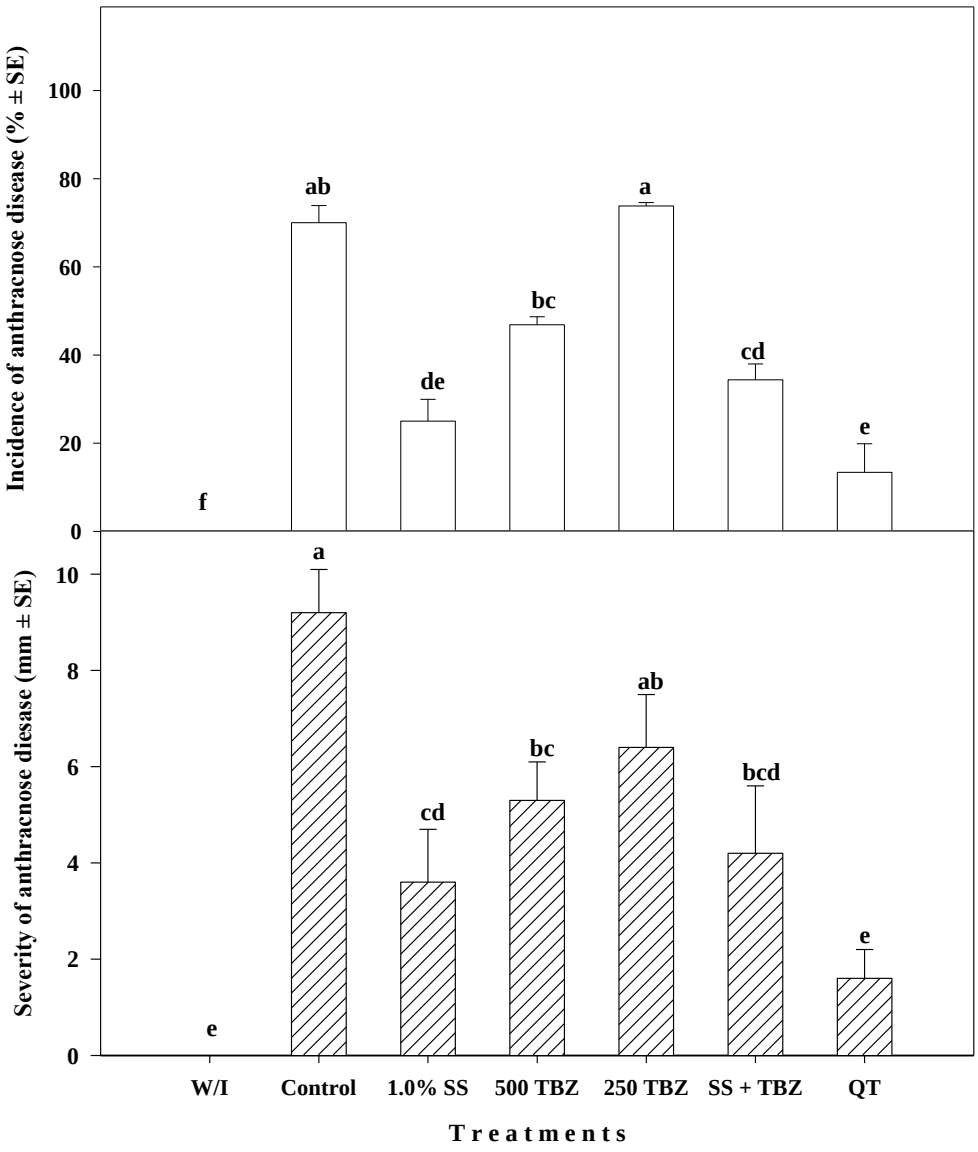

Figure 4.6. Effectiveness of 1.0% sodium silicate alone (SS), 250 ppm fungicide thiabendazole (250 TBZ), 500 ppm thiabendazole (500 TBZ), combination of 1.0% SS and 250 ppm TBZ (SS + TBZ) and quarantine treatment (48°C for 20 min; QT) to control anthracnose disease on papayas cv. Maradol artificially inoculated with *C. brevisporum*, treated 1-2 h before for 90 s at 20°C, and incubated for 5 d at 25-26°C and 80-90% RH.

4.5. Discussion

It has been determined in this work the antifungal effect of postharvest Si treatments in its form of potassium and sodium silicate against postharvest papaya anthracnose disease. Firstly, we have conducted an *in vitro* study in order to take like reference the most effective concentration or concentrations of Si added on PDA medium to inhibit the mycelial growth of *C. brevisporum*. Ranges of concentration of 1.5-3.0 PS and 2.0-3.0 % SS, respectively (Fig. 4.1), were taken like reference, to be used in further *in vivo* primary screenings. Secondly, we have conducted an *in vivo* preliminary study in order to select the most effective concentration of Si to inhibit the development of postharvest anthracnose disease on before (curative treatment) or after (preventive treatment) inoculated papayas. The concentrations of 1.5 % PS and 1.0 % SS were chosen, among the wide range of concentrations tested (Fig. 4.3), to be used in further small-scale trials. Thirdly, an experiment was designed to determine the dip times in PS or SS aqueous solutions, and dip times for 60 and 90 s were selected for PS and SS, respectively, since a practical and not statistical point of view. Finally, an experiment was conducted to determine the effectivity of Si alone or combined with low doses of thiabendazole fungicide on anthracnose disease.

Regarding the effectivity of Si in its form of PS to inhibit the mycelial growth of *C. brevisporum* under *in vitro* conditions. In general, we have found that the concentrations of 3.0 y 2.5 % PS were the most effective (93 and 91 % of inhibition of mycelial growth, respectively), after 9 d of incubation at 25 °C (Fig. 1). Our results obtained with potassium silicate were lightly minor to those obtained by Bekker *et al*. (2009) with potassium silicate at 20 mL L^{-1} (~ 2.0 %; 100 % of inhibition of *C. gloeosporioides*) and to those reported by Bautista-Baños *et al*. (2003) who found that Chitosan at 2.0 and 3.0 % inhibited completely

the mycelial growth of *C. gloeosporioides*. Likewise, Ali and Mahmud (2008) revealed that Chitosan at 2.0 % inhibited totally the mycelial growth of *C. gloeosporioides*. In contrast, our findings were highly superior to those reported by de Oliveira (2017) in where it were observed 30.0 and 35.8 % of inhibition of mycelial growth for *C. fructicola* and *C. tropicale*, respectively, isolated of mango fruit when applied Chitosan (5 mg mL^{-1}; ~ 0.5 %).

Regarding the effectivity of Si in its form of SS to inhibit the mycelial growth of *C. brevisporum* under *in vitro* conditions, we have found that the 2.5 and 3.0 % concentrations of SS suppressed completely the radial mycelial growth of the fungus after 9 d of incubation at 25 °C (Fig. 4.1). These results of reduction of mycelial growth of *C. brevisporum* were superior to those reported by Ge *et al*. (2017) where SS at 2.4 % did not suppress completely the mycelial growth of *Trichothecium roseum* in the same conditions of temperature and incubation period. In contrast, other studies have revealed a more effective control to reduce the mycelial growth of various postharvest fungi using low doses of SS. For instance, Liu *et al*. (2010) mention that 0.5 % of SS were sufficient to suppress completely the mycelial growth of *P. digitatum* isolated of citrus diseased fruits, and Elsherbiny and Taher (2018) reported that 10 mM (~ 0.28 %) of sodium metasilicate inhibited the mycelial growth of *Sclerotia sclerotiorum* (92.2 % of inhibition after 6 d of incubation). Likewise, dos Passos Braga *et al.* (2019) found that 7.5 mg mL^{-1} (~ 0.75 %) of Chitosan were sufficient to inhibit totally the mycelial growth of three isolates of *C. brevisporum* isolated of diseased papaya fruit with anthracnose

Regarding the curative effectivity of Si to reduce the incidence of postharvest anthracnose in *in vivo* primary screenings, we have generally found that the treatments with PS were not effective in all the tested concentrations of PS, after 6 d of incubation at 25-26 °C (Fig. 2). In

contrast, Moscoso-Ramírez and Palou (2014) found that 90 mM (~ 1.4 %) potassium silicate significantly reduced the incidence of blue mold in citrus fruits (40 % of incidence reduction) in curative treatment. We consider that the type of pathogen is playing an important role on the curative effectivity of Si, depending on of whether the fungus is of wound or latent. In this case very particular, *C. brevisporum* is a latent fungus, whereas *Penicillium italicum* is a wound fungus. Our unsatisfactory results with Si applied in curative treatment can be supported by Bautista-Baños *et al*. (2003) who found that Chitosan at 1.5 % dipped for 20 min did not significantly reduce the papaya anthracnose incidence in curative treatments.

With regards to the preventive effectivity of Si to reduce the incidence of postharvest anthracnose in *in vivo* primary screenings, our results show that 1.5 % PS and 1.0 SS were highly effective (6.3 and 0.0 % of incidence, respectively) among all the tested concentrations of PS and SS, after 6 d of incubation at 25-26 °C (Fig. 4.3). The trend of these results obtained in this study showed that either PS or SS treatment inhibited anthracnose development in a concentration-dependent manner, and they were more effective at a concentration of 1.5 and 1.0 %, respectively. Therefore, both PS and SS acted here probably more as a plant growth regulator, which are typically more effective when applied at an optimal concentration, than as a conventional fungicide, whose efficacy gradually increases as the application dose increases. This hypothesis was supported by the fact that in the screenings for preventive action, disease reduction was lower after PS and SS application at 2 and 1.5 % than at 1.5 and 1.0 % (Fig. 4.3). Moreover, in the case of PS, aqueous PS was also included in the list of synthetic substances allowed for use in organic crop production. The only requirement is that the silica used for the manufacture of PS must be sourced from naturally occurring sand (USDA AMS 2010). Our results are superior to several reported studies using Si in other postharvest

diseases. For instance, Moscoso-Ramírez and Palou (2014) revealed an incidence reduction of blue mold of 52 % when applied 90 mM (~ 1.4 %) potassium silicate in citrus fruits after 6 d of incubation at 20 °C. Moreover, Liu *et al*. (2010) reported that 0.5 % SS reduced up to 45 % the incidence of green mold in citrus fruit.

Regarding the time of preventive dip treatments with Si, our findings showed that there was not significant differences among them (Fig. 4.4), but the times of preventive dip of 60 s and 90 s were selected for PS y SS since a practical and not statistical point of view based on the values of the anthracnose incidence, with values of incidence of 6.7 and 0.0 %, respectively, as part of the characterization of the dip treatments. Thus, anthracnose development on Si-treated papayas was not significantly affected by immersion time. Our findings with these treatments with Si in aqueous solutions were superior to earlier research works such as the reported by Sivakumar *et al*. (2002) in where the ammonium carbonate at 3.0 %, amended to wax formulation significantly reduced the papaya anthracnose incidence by 70 %. Our findings with these treatments with Si in aqueous solutions were superior to those obtained by Moscoso-Ramírez and Palou (2014) who reported an incidence reduction of green mold by 37 % in citrus fruits stored at 20 °C for 6 d. Likewise, our results with Si in aqueous solutions were superior to other low-toxicity substances used against postharvest papaya anthracnose. For example, Bautista-Baños *et al*. (2003) found that Chitosan at 1.5 % dipped for 20 min reduced the papaya anthracnose incidence until a 40 % in preventive treatments. Vilaplana *et al*. (2020) found that the treatment of hot water (49 °C for 20 min) combined with different concentrations of chitosan coating (0, 5, 10, 15 and 20 g L^{-1}; ~ 0, 0.5, 1.0, 1.5 and 2.0 %) was not capable of reducing the incidence of papaya anthracnose, but whether the severity, after 28 d of storage at 10 °C plus 7 d at 20 °C in papayas inoculated with *C. fructicola*. Moreover,

Ayón-Reyna *et al*. (2017) reported that the anthracnose incidence on papaya fruit was controlled only until 12 d after application of the combined treatment of hot water (48 °C) with calcium chloride (1.0 %) during 20 min in curative treatments, but whether reduced the severity, during the storage at 12 °C for 20 d in papayas inoculated with *C. gloeosporioides*.

Similarly, dos Passos Braga *et al*. (2019) revealed that the treatment of 5 mg mL^{-1} (~ 0.5 %) Chitosan combined with *Menta piperita* L., essential oil 1.25 µL mL^{-1} (~ 0.12 %) applied in coating on papaya fruit did not reduce the incidence of papaya anthracnose, although the anthracnose severity was reduced by 75, 58 and 100 % for the isolates CMM 1936, CMM 1642 and 1728 of *C. brevisporum* inoculated artificially after treatment during 10 d of storage at 25 °C.

It is believed that the mode of action of Si treatments is through a direct effect on the fungi spores. For instance, Liu *et al*. (2010) reported damage on the plasma membrane of Si-treated *P. digitatum* spores, leading to higher leakage of proteins and sugars. However, other research works contradicted this hypothesis and attributed the antifungal action of Si to indirect effects to the fruit host. Hereto, Si can play an important role on the formation of physical and mechanical barriers to the penetration of pathogens at the cell wall level (Datnoff et al., 2001; Buonaurio *et al*., 2009). Si treatments may also act by eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds and pathogenesis-related (PR) proteins in infected plants (Epstein, 1999). According to our satisfactory results in preventive treatments in this study, we can hypothesize that in the case of papaya *C. brevisporum* anthracnose, the mode of action of postharvest PS and SS treatments for disease control might be an indirect effect on the fruit host, forming physical and mechanical barriers at the cell wall level.

Finally, our results on the effectivity of Si alone or combined with low doses of thiabendazole fungicide on postharvest anthracnose disease have revealed that the Si combined with thiabendazole fungicide lacks of an synergistic effect because its combination did not improve the control of postharvest anthracnose disease on papaya cv. Maradol (Fig. 4.5 and 4.6). These findings differ of those reported by other researchers in where indicate the presence of a synergistic effect between two substances such as hot water plus Chitosan (Vilaplana *et al*., 2020), hot water plus calcium chloride (Ayón-Reyna *et al*., 2017) and Chitosan plus *Menta piperita* L., essential oil (dos Passos Braga *et al*., 2019).

The primary findings of this research work were that postharvest Si treatments showed significant preventive antifungal activity against papaya *C. brevisporum* anthracnose. These findings indicate that Si can be tested to prevent fruit anthracnose in pre harvest in future research works, considering that *Colletotrichum spp* cause latent infections, very difficult of controlling in curative treatments. To our knowledge this is the first research report of Si on fungal papaya postharvest anthracnose. Considering that Si is the second most abundant atom in the earth's crust and it is readily available, the cost of Si treatments are relatively inexpensive and, in any case, will be lower than that of other new alternative strategies for papaya postharvest disease control. Although large-scale semi-commercial trials are needed for efficacy assessment before commercial implementation, Si treatments show great potential as part of non-polluting integrated disease management programs.

4.6. Acknowledgements

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CAPÍTULO V. CONCLUSIONES GENERALES

La antracnosis poscosecha en papaya Maradol en México es causada por *Colletotrichum brevisporum*, siendo éste el primer reporte de su presencia.

El Silicio a 3.0 y 2.5% inhibió el crecimiento micelial radial de *C. brevisporum*, por arriba del 90%, bajo condiciones *in vitro*.

El silicio no mostró efecto curativo significativo sobre la antracnosis poscosecha en papaya Maradol en experimentos primarios *in vivo*.

El SP a 1.5% y SS a 1.0% redujeron significativamente la incidencia de la antracnosis por arriba del 93%, mientras que sólo SS a la dosis mencionada redujo consistentemente la severidad, mediante experimentos primarios preventivo *in vivo* y estas dosis fueron seleccionada para usarlos en tratamientos de baños preventivo.

Los tiempos de inmersión 30, 60, 90 y 180 s, cuando SP a 1.5% y SS a 1.0% fueron aplicados, respectivamente, a 20°C, no influenciaron la efectividad en el control de la antracnosis.

El silicio aplicado en baño, combinado con bajas dosis de tiabendazol no mejoraron la efectividad del control de la antracnosis en frutos de papaya Maradol.