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DESARROLLO DE UN PRODUCTO BIOTECNOLÓGICO CON APLICACIÓN INHIBITORIA PARA BACTERIAS FITOPATÓGENAS DEL CULTIVO DE TOMATE.

Tesis

Que presenta ROBERTO ARREDONDO VALDÉS

Como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN PARASITOLOGÍA AGRÍCOLA

Saltillo, Coahuila Junio 2017

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Elaborada por ROBERTO ARREDONDO VALDÉS como requisito parcial para obtener el Grado de Doctor en Ciencias en Parasitología Agrícola con la supervisión y aprobación del Comité de Asesoría.

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Introducción

El tomate (*Solanum licopersycum* L.) es la hortaliza más difundida en el mundo y la de mayor valor económico tanto por la superficie que se cultiva así como por la producción obtenida, ya que ocupa el segundo lugar después de la papa; además la alta remuneración económica, generación de empleos y propiedades nutricionales. La superficie cultivada del tomate se estima en más de 3 millones de ha en el mundo, con una producción promedio anual de 126.2 millones de toneladas. Su demanda aumenta continuamente y con ella su cultivo, producción y comercio. En México se cultivan alrededor de 64 mil hectáreas anuales de tomate. El cultivo del tomate ocupa el primer lugar en exportación, desde hace 20 años, sin embargo; entre los factores que inciden negativamente en la productividad del cultivo de tomate resaltan las enfermedades causadas por hongos, bacterias y virus. Entre las bacterias que afectan al cultivo se encuentra a *Agrobacterium tumefaciens* (Agalla de corona) *Clavibacter michiganensis* subsp *michiganensis* (cancro bacteriano), *Pseudomonas syringae* pv *tomato* (mancha negra), *Ralstonia solanacearum raza 3* (marchitez bacteriana), y *Xanthomonas axonopodis pv vesicatoria* (mancha bacteriana). Problemas por los que se han estudiado métodos de diagnóstico y control de dichas enfermedades. Algunos de los métodos de control son el control cultural, la aplicación de antimicrobianos químicos, entre otros, sin embargo; los métodos mencionados presentan ciertas desventajas. Los antibióticos químicos representan un riesgo para el medio ambiente y para la salud, otros son muy costosos. Por las razones anteriores es que se han buscado alternativas que no involucren daño al ambiente o a la salud, siendo una área de oportunidad la búsqueda en extractos naturales de plantas, por ejemplo *Lippia graveolens*, posee compuestos volátiles, lípidos y fenólicos con diferentes propiedades empleados a nivel industrial, como fragancia en jabones, perfumes, cosméticos, saborizantes; además se le atribuyen propiedades antibacteriales, antifúngicas, antiparasitarias, antimicrobianas y antioxidantes. México ocupa el segundo lugar como productor mundial de *Lippia graveolens* siendo esta planta de gran perspectiva para su empleo en el control de las bacterias del cultivo de tomate. Por su parte *Larrea tridentata*

posee ceras complejas, compuestos volátiles, saponinas, triterpenos, ácido nordihidroguaireico, así como un gran número de compuestos fenólicos y glicósidos; en la literatura esta planta se usa en el tratamiento de diversos padecimientos como: reumatismo, problemas renales, dolor de cabeza, gastritis, diarrea, parásitos, enfermedades venéreas, escalofríos, tos, indigestión y tuberculosis; otros usos que se le atribuyen son su capacidad antimicrobiana y antifúngicas. Uno de los principales usos de *Agave lechuguilla* en México es la obtención de la fibra, sin embargo en la actualidad se han realizado estudios de efectos antitumorales, antibacteriales y antifúngicos debido a sus componentes fitoquímicos entre los cuales se encuentran alcaloides, triterpenos, esteroides, azucares reductores, fenoles, taninos, aminoácidos libres y saponinas. A la planta *Jatropha dioica* se le atribuyen actividades antimicrobianas, sin embargo; hay poca información sobre su composición fitoquímica; de las raíces se obtiene un aceite esencial, resina, saponinas, un alcaloide y ácido oxálico. *Carya illinoinensis* por su parte se caracteriza por poseer actividad antiséptica, antimicrobiana o propiedades regenerativas. Por otro lado la mirada de los investigadores se dirige hacia los microorganismos, que pueden ser usados como agentes de control biológico (CB); *Bacillus subtilis* posee metabolitos que han sido reportados como promotores de crecimiento o agentes de biocontrol, entre el grupo de las hormonas que estimulan el crecimiento de la planta se encuentran las auxinas y las citoquininas; además *Bacillus sutilis* tiene la capacidad de solubilizar fosfatos presentes en el suelo, por medio de formación de ácidos orgánicos, producidos por la degradación de carbohidratos. *Bacillus subtilis* es capaz de producir más de dos docenas de antibióticos con una increíble variedad de estructuras. Mientras tanto, para *Trichoderma ssp.* se reporta la inducción de resistencia en las plantas al estimular los mecanismos naturales de defensa de estas, al mejorar la producción de hormonas, peroxidasas y compuestos fenólicos, por otro lado las tichorziaminas se reportan como metabolitos secundarios peptídicos con efectos antibióticos. El presente trabajo pretende probar, *in-vitro* e *in-vivo*, la actividad inhibidora de crecimiento y antimicrobiana de extractos naturales obtenidos bajo diferentes procedimientos, utilizando como fuentes de extractos plantas y bacterias de control

biológico con antecedentes antimicrobianos que se encuentren en la región norte de México, para formular un producto biotecnológico con la capacidad de proteger el cultivo de tomate.

Justificación

El jitomate es originario de la América del Sur, de la región andina, particularmente de Perú, Ecuador, Bolivia y Chile. Sin embargo, su domesticación fue llevada a cabo en México. En el 2008, China fue el principal productor de jitomate en el mundo, con una participación de 36%, le sigue Estados Unidos con 14%; Turquía, 12%; India, 11%; mientras que México ocupó el doceavo lugar, con 3% de participación en la producción mundial. Los países que ocupan los primeros tres lugares en el lugar de mayores exportadores, comercializan poco más de 55% de total mundial. Holanda ocupa el primer sitio, con 22% del volumen de exportaciones mundiales de jitomate; México tiene el segundo lugar con 18% de las mismas; en tercer lugar, España con 17% del total mundial. Dentro del mercado estadounidense, el 80% de las importaciones de jitomate son de origen mexicano. El tomate representa uno de los principales alimentos a nivel mundial, y además es de importancia económica en países como EUA, Canadá, Alemania, Francia y México. Razón por la cual el desarrollo de métodos de control de las principales bacterias fitopatógenos del tomate es de gran importancia. Existen antimicrobianos ampliamente aplicados; sin embargo los efectos adversos que estos presentan a la salud humana y al ambiente son elevados, por lo que en la actualidad se buscan alternativas más naturales y por ello con menos riesgos a la salud y el ambiente. En el presente estudio se aplicaran extractos de la plantas *Lippia graveolens*, *Larrea tridentata, Agave lechuguilla, Jatropha dioica* y *Carya illinoinensis* de la región semidesértica del norte de México; así como extractos de *Bacillus ssp Trichoderma ssp*, para inhibir las enfermedades ocasionadas por *Clavibacter michiganensis* subsp *michiganensis*, *Pseudomonas syringae pv tomato*, *Xanthomonas axonopodis pv vesicatoria* y *Ralstonia solanacearum raza 3* en los cultivos de tomate. Los mejores extractos

obtenidos serán formulados en un producto biotecnológico para inhibir estas bacterias en el cultivo de tomate.

Hipótesis

Existen componentes fitoquímicos de *Agave lechuguilla, Carya illinoinensis, Jatropha dioica, Larrea tridentata, Lippia graveolens, Origanum vulgare*, así como metabolitos de *Bacillus spp.* y *Trichoderma spp.*, con capacidad antibacteriana para formular un producto biotecnológico en el cultivo de tomate.

Objetivos

General

Desarrollar un producto biotecnológico con posible aplicación inhibitoria para bacterias fitopatógenas del cultivo de tomate.

Específicos

- Obtener y comparar extractos crudos contra resuspendidos de las plantas *Agave lechuguilla, Carya illinoinensis, Jatropha dioica, Larrea tridentata, Lippia graveolens, Origanum vulgare,* así como extractos de metabolitos secundarios *Bacillus spp.* y *Trichoderma spp.* para el control bacteriano del cultivo de tomate.
- Realizar análisis de compuestos fitoquímicos a los extractos de planta y metabolitos secundarios bacterianos obtenidos.
- Determinar la actividad antibacteriana de los extractos de plantas y de metabolitos de las bacterias, mediante técnica de microplaca
- Analizar y seleccionar los extractos con mejor actividad antibacteriana mediante espectrofotometría-IR.

- Evaluar los extractos obtenidos *in-vivo* en cultivo de tomate en base a incidencia y severidad de las enfermedades causadas por *Agrobacterium tumefaciens, Clavibacter michiganensis* subsp *michiganensis*, *Pseudomonas syringae pv tomato*, *Xanthomonas axonopodis pv vesicatoria* y *Ralstonia solanacearum raza 3*.

Revisión de literatura

Cultivo de Tomate

Descripción e importancia

El tomate es una de las hortalizas más importantes en el mundo tanto por la superficie que se cultiva así como por la producción obtenida, ya que ocupa el segundo lugar después de la papa; además de la alta remuneración económica, generación de empleos y propiedades nutricionales (Ramírez y Sáinz, 2006). La superficie cultivada del tomate se estima en más de 3 millones de hectáreas en el mundo, con una producción promedio anual de 126.2 millones de toneladas; actualmente el tomate se cultiva en más de 160 países siendo los principales productores China, Estados Unidos, Turquía, India, Italia, Egipto, España, Brasil, Irán y México (FAO Statics, 2007). El tomate es la hortaliza más difundida en el mundo y la de mayor valor económico. Su demanda aumenta continuamente y con ella su cultivo, producción y comercio. El incremento anual de la producción en los últimos años se debe principalmente al aumento en el rendimiento y en menor proporción de la superficie cultivada. En México se cultivan alrededor de 64 mil hectáreas anuales de tomate, de las que se obtiene una producción de alrededor de 2,425000 toneladas (SIAP-SAGARPA, 2008). El tomate se cultiva en la mayoría de los estados, cabe destacar al estado de Sinaloa como el principal productor con el 35% de la producción nacional, seguido de Baja California, San Luís Potosí, Zacatecas, Nayarit, Michoacán, Sonora, Veracruz, Morelos y Puebla. El tomate ocupa el primer lugar en exportación, desde hace 20 años, sin embargo entre los factores que inciden negativamente en la productividad del cultivo de tomate

resaltan las enfermedades causadas por hongos, bacterias y virus (Ramírez y Saínz, 2006). Entre las bacterias que afectan al cultivo se encuentra a *Clavibacter michiganensis* subsp *michiganensis* (cancro bacteriano) (Flores *et al.,* 2009), *Pseudomonas syringae pv tomato* (peca bacteriana) (Latorre, 2011), *Xanthomonas axonopodis pv vesicatoria* (mancha bacteriana) (Flores, 2008) y *Ralstonia solanacearum raza 3* (marchitez bacteriana) (Feliciano y Zubiaur, 2013).

Principales enfermedades bacterianas del tomate

Agrobacterium tumefaciens

El género *Agrobacterium* agrupa a la bacteria fitopatógena *A. tumefaciens*, causante de tumores en cuello y raíces (Crown gall) en muchas especies vegetales, de interés agrícola y comercial. De Cleene y De Ley (1976) recopilando datos de la bibliografía y otros obtenidos en sus propios ensayos, concluyeron que en total 643 especies de 331 géneros, pertenecientes a 93 familias, eran susceptibles a esta bacteria. En los viveros e invernaderos es donde más daños se producen, al aparecer episodios epidémicos que pueden impedir la comercialización de producciones enteras, porque las plantas afectadas por esta enfermedad no pueden ser comercializadas. Los estudios epidemiológicos y de dinámica de poblaciones del patógeno son poco abundantes y han incidido en aspectos del desarrollo y mantenimiento de la enfermedad producida por la bacteria.

Clavibacter michiganensis subsp *michiganensis*

Esta bacteria es un bacilo Gram positivo, no móvil, aeróbico, productor de capsula, cuya temperatura optima de crecimiento *in-vitro* es de 25 a 28 °C (Schaad *et al.,* 2000), la bacteria se transmite por semilla infectada y posteriormente penetra a los tejidos vasculares a través de heridas, estomas, tricomas, e hidtidés de la hoja (Flores *et al.,* 2009). El marchitamiento marginal de foliolos es uno de los primeros

síntomas en plantas de todas las edades, posteriormente aparecen estrías necróticas que se extienden desde la parte inferior del peciolo hasta el punto que se une con el tallo, ya que la bacteria es un invasor sistémico de los tejidos del floema, medula y corteza. Finalmente la planta se necrosa y se marchita, y es característico que los bordes de foliolos inferiores aparezcan secos y curvados hacia abajo, y que luego adquieran un color castaño y necrótico con el peciolo unido al tallo. Bajo ciertas condiciones, las manchas necróticas se abren y forma cancros, como fuente de infecciones secundarias. Las plantas enfermas en el fruto forman pequeñas mancha necróticas rodeadas de un halo claro, que se conoce como “ojo de pájaro” (Blancar, 1996), el cancro bacteriano se ha dispersado en el mundo y ha causado pérdidas serias de las cosechas en invernadero y en campo en Canadá se han registrado perdida de producción de 20% o más, en Francia de 30%(OEPP/EPPO, 2005) y en EUA de más del 46% (Flores *et al.,* 2009).

Pseudomonas syringae pv tomato

En 1986 aparece el primer reporte de *Pseudomonas syringae* pv. Tomato, esta bacteria puede afectar a las hojas, tallos y frutos. En las hojas aparecen pequeñas lesiones necróticas, preferentemente en los márgenes y siempre rodeadas por un prominente halo clorótico. Estas lesiones pueden confluir comprometiendo gran parte del foliolo, con lo cual la planta adquiere un aspecto atizonado. En frutos inmaduros se desarrollan pequeñas lesiones necróticas sub-superficiales, generalmente de color pardo (peca bacteriana) (Latorre, 1990). Las lesiones negras con bordes amarillos también pueden ocurrir en los márgenes de las hojas donde se acumulan las gotas por efecto del proceso de gutación; cuando estas lesiones se unen, pueden morir grandes áreas de tejido foliar. Generalmente las lesiones en el fruto son pequeñas (1mm), con forma de lunar y superficiales; sin embargo, también pueden ser más grandes y heridas, y en frutos inmaduros están rodeadas por un halo verde (Corpeño, 2004). El agente causal puede penetrar por heridas, estomas y que se puede transmitir por semilla contaminada y a través del suelo. Se

dispersa por efecto del salpicado producido por la lluvia o por el sistema de riego por aspersión. Posiblemente sea movilizada a largas distancias en semillas infectadas (Latorre, 1990). Además, puede sobrevivir en la rizósfera y en restos vegetales, en forma epifita en tomate y malezas (Dotson *et.al.,* 2007).

***Ralstonia solanacearum* raza 3**

La marchitez bacteriana de las solanáceas causada por *Ralstonia solanacearum* es una de las enfermedades más importantes en plantas, está presente en el mundo en regiones tropicales, subtropicales y templadas. La bacteria invade a las plantas hospederas a través de la raíz y coloniza los vasos del xilema en el sistema vascular. Las plantas infectadas con *Ralstonia solanacearum* muestran disminución en el crecimiento, amarillamiento, marchitez repentina en las hojas más jóvenes durante las horas más calurosas del día. Por la noche, con las temperaturas frescas, las plantas enfermas recuperan su turgencia, hasta que llegan a la etapa de marchitez permanente debido al taponamiento de los tejidos vasculares (García, 2013).

Xhantomonas axonopodis pv vesicatoria

El patógeno *Xhantomonas axonopodis pv vesicatoria* causa la enfermedad de la mancha bacteriana en tomate, está ampliamente distribuida en los campos agrícolas de todo el mundo, la infección se propaga a partir de semillas y su dispersión ocurre de manera mecánica por manejo o irrigación. Los frutos presentan manchas superficiales ovaladas e irregulares en forma de círculos de 2-10 mm de diámetro, mientras que las hojas presentan lesiones irregulares de coloración verde tornándose café. (Flores, 2008). Síntomas similares pueden ser causados por *C. michiganensis subsp. michiganensis y Alternaria solani* por lo que

es necesario realizar aislamientos de la bacteria en cultivos *in vitro* para su eficiente diagnóstico.

Control de enfermedades del cultivo del tomate

Control cultural

El control cultural consiste en la utilización de las prácticas agrícolas ordinarias, o algunas modificaciones de ellas, con el propósito de contribuir a prevenir los ataques de los insectos, hacer el ambiente menos favorable para su desarrollo, destruirlos, o disminuir sus daños. La adecuada aplicación de las prácticas agrícolas con estos fines, requiere de conocimientos apropiados sobre la fisiología y fenología de las plantas cultivadas y de sus características agronómicas; de las modalidades de las prácticas agrícolas propiamente dichas; y naturalmente, un buen conocimiento de la biología de las plagas locales, su comportamiento y su ocurrencia estacional. La aplicación de prácticas culturales inadecuadas, derivadas del desconocimiento de los factores antes mencionados, puede conducir al agravamiento de los problemas fitosanitarios. Para 1990 la FAO propone para el problema ocasionado por *Ralstonia Solanacearum* sembrar semilla sana, plantar en suelos sin antecedentes de presencia de esta bacteria, usar agua de riego sin contaminar, rotar la papa con cultivos no hospederos de esta bacteria como ajo o cebolla, asegurar buen drenaje del terreno, controlar plagas de suelo que pueden ayudar a la diseminación e infección y desinfectar el equipo agrícola con hipoclorito de sodio al 2%, yodo agrícola o amonios cuaternarios. Por su parte la FAO propone para *Pectobaterium caratovora e*vitar humedad excesiva en la cosecha, almacenar tubérculos secos y mantener la semilla en condiciones de buena aireación, evitando que se humedezca. Sin embargo aunque el control cultural representa una prevención de las enfermedades en ocasiones se emplean desinfectantes como sulfato de gentamicina y clorhidrato de oxitetraciclina, siendo esto un riesgo a para que las bacterias desarrollen resistencia a estos antibióticos; por esto mismo en la

actualidad se realiza una búsqueda por componentes preventivos de origen natural para el control de enfermedades causadas por bacterias fitopatógenas tales como: *Xanthomonas axonopodis pv. phaseoli* que ocasiona el tizón común del fríjol, *Xanthomonas axonopodis pv. Vesicatoria* que ocasiona mancha bacteriana; *Erwinia carotovora subsp. carotovora* causante de la enfermedad pudrición blanda y *Pseudomonas cichorii* que ocasiona la bacteriosis de la lechuga.

Control químico

Durante muchos años, una gran variedad de productos químicos y compuestos sintéticos han sido utilizados como agentes antimicrobianos para inhibir la acción de los fitopatógenos. Productos químicos antimicrobianos, como los benzimidazoles, hidrocarburos aromáticos y los inhibidores de la biosíntesis de los esteroles, se utilizan a menudo en el control de enfermedades de las plantas en la agricultura. Sin embargo, hay una serie de problemas en contra del uso eficaz de estos productos químicos en áreas donde los hongos han desarrollado resistencia. Para superar este problema, mayores concentraciones de estos químicos aumentan el riesgo de residuos tóxicos de alto nivel en los productos (Tripathi y Dubey, 2004). Algunos productos comerciales que se usan actualmente se presentan en la Tabla 1.

Tabla 1. Productos químicos aplicados en enfermedades del cultivo de tomate en la agricultura mexicana.

Enfermedades y plagas del cultivo de tomate	Control
Mosca Blanca *Trialeurodes vaporariorum* y *Bemisia tabaco*	Una amplia gama de piretroides (cipermetrín, deltametrín, fenpropatrín, fluvalinato, bifentrín, permetrín, alfacipermetrín, cihelatrínlambda, ciflutrín) despolarización membranal, inducen picos de señalización en nervios sensoriales y motores.
Trips de las Flores *Franklinella occidentalis*	Formetanato, aceite de verano, metiocarb, fenitrotión, malatión, naled y acrinatrin, inhibición de la conducción de acetil colinesterasa.
Ácaro Rayado o Arañuela roja *Tetranychus urticae*	Activador del canal de cloro, Abamectina; Inhibidores de crecimiento de los ácaros, Clofentezin,Hexitiazox, Etoxazol; Inhibidores del transporte de electrones punto, Piridaben

	Tebufenpirad, Fenpiroximato; Inhibidores de la síntesis ATP mitocondrial, Propargita Inhibidores de la síntesis de los lípidos, Spirodiclofen.
Ácaro bronceado del tomate ***Aculops lycopersici***	La abamectina es el producto de la fermentación natural de esta bacteria. Insecticida de acción translaminar y sistemia localizada, de amplio espectro. Actúa estimulando la liberación presináptica del inhibidor neurotransmisor ácido γ-aminobitírico desde las terminales nerviosas y potenciando la fijación de este ácido a los receptores postsinápticos, entre ellos el receptor glutamato.
Palomilla del Tomate ***Tuta absoluta***	Piretrinas y piretroides se debe a su acción sobre la bomba de sodio de las neuronas. Mediante un proceso fisicoquímico estas moléculas inhiben el cierre del canal de sodio de la membrana celular, de manera que producen una transmisión continua del impulso nervioso. Las consecuencias de esta transmisión continua son los temblores, la parálisis muscular (llamado "efecto derribo" o "knock-down", característico de las piretrinas II) y, eventualmente, la muerte (específica de las piretrinas I).
Perforador del fruto de tomate ***Neoleucinodes elegantallis***	Intrepid, Actúa sobre la fase de larvas de las plagas.imitando la hormona natural de la muda del insecto (hidroxiecdisona) induciendo a la larva a una muda prematura letal, impidiendo deshacerse de su vieja cutícula por lo que muere de deshidratación e inanición después de ingerir el tejido vegetal tratado. Match, s un insecticida regulador del crecimiento de los insectos que interfiere con la síntesis de la quitina. Su modo de acción es específico para artrópodos, inhibiendo el crecimiento de larvas, especialmente de lepidópteros y de coleópteros. En algunos insectos actúa también como ovicida.
Mal de los almácigos o Damping off ***Rhizoctonia solani, Pythium spp., Fusarium spp., Sclerotinia spp., Sclerotium spp. y Phytophthora spp***	Dimetomorf: (E,Z)-4-[3-(4clorofenil)-3-(3,4-dimetoxifenil)acriloil) morfolina Fungicida de acción preventiva y antiesporulante para el control de la enfermedad causada por *Phytophthora infestans* en los cultivos de papa y tomate Tiofanato metílico: Dimetil-4,4-0-Fenilenbis (3-tioalofanato) Contra los hongos: *Monilinia spp., Botrytis spp., Sclerotinia spp., Fusarium oxysporum, Sclerotinia sclerotiorum,Gloeosporium spp*.
Moho gris ***Botrytis cinerea***	Tiofanato metílico: Dimetil-4,4-0-Fenilenbis (3-tioalofanato) Contra los hongos: *Monilinia spp., Botrytis spp., Sclerotinia spp., Fusarium oxysporum, Sclerotinia sclerotiorum,Gloeosporium spp*.
Oidio ***Leveillula taurica o Erysiphe spp***	Azoxystrobin, Su mecanismo de defensa se basa en la secreción de dos sustancias, la estrobilurina A y la oudemansina A. Estas sustancias les permiten mantener a sus competidores a distancia y matarlos cuando están a su alcance.
Moho de las hojas ***Cladosporium fulvum***	Mancozeb no-sistémico de ditiocarbamato con acción protectora multi-sitio, en contacto. Es una combinación de otros dos ditiocarbamatos: maneb y zineb, Reacciona con, e inactiva, los grupos sulfhidrilo de aminoácidos y enzimas de células fúngicas, resultando en la interrupción del metabolismo lipídico , la respiración y la producción de trifosfato de adenosina
Fusariosis ***Fusarium oxysporum***	Tiofanato metílico: Dimetil-4,4-0-Fenilenbis (3-tioalofanato) Contra los hongos: *Monilinia spp., Botrytis spp., Sclerotinia spp., Fusarium oxysporum, Sclerotinia sclerotiorum,Gloeosporium spp*.
Tizón temprano ***Alternaria dauci***	zineb, maneb, clorothalonil El clorotalonil reduce las moléculas de glutatión intracelular de hongos a formas alternativas que no pueden participar en las reacciones enzimáticas esenciales, lo que en última instancia conduce a la muerte celular, similar al mecanismo del triclorometilsulfenilo.
Tizón tardío ***Phytophthora infestans***	Metalaxyl+clorotalonil, causa ruptura de la glicólisis y producción de energía. El inhibe la esporulación de los hongos pues evita su instalación al inhibir la respiración del mismo, la biosíntesis de aminoácidos, la metabolización de uridina y al afectar la permeabilidad celular.
Tizón ***Phytophthora capsici***	Tris [amino-[etileno bis (ditiocarbamato)] de zinc (II)][tetrahidro-1,2,4,7 - ditiadiazocina-3, 8-dition] polímero Fungicida contra: Tizón tardío (*Phytophora infestans*), Tizón temprano (*Alternaria solani*), Cenicilla (*Podosphaera leucotricha*) y Roña o sarna (*Venturia inaequalis*).
Podredumbre del tallo y raíz ***Phytophthora parasitica***	Tris [amino-[etileno bis (ditiocarbamato)] de zinc (II)][tetrahidro-1,2,4,7 - ditiadiazocina-3, 8-dition] polímero Fungicida contra: Tizón tardío (*Phytophora infestans*), Tizón temprano (*Alternaria solani*), Cenicilla (*Podosphaera leucotricha*) y Roña o sarna (*Venturia inaequalis*).

Podredumbre húmeda del tallo – Moho blanco ***Sclerotinia sclerotiorum***	Tris [amino-[etileno bis (ditiocarbamato)] de zinc (II)][tetrahidro-1,2,4,7 - ditiadiazocina-3, 8-dition] polímero Fungicida contra: Tizón tardío (*Phytophora infestans*), Tizón temprano (*Alternaria solani*), Cenicilla (*Podosphaera leucotricha*) y Roña o sarna (*Venturia inaequalis*).
Mancha gris de la hoja ***Stemphylium spp***	Mancozeb no-sistémico de ditiocarbamato con acción protectora multi-sitio, en contacto. Es una combinación de otros dos ditiocarbamatos: maneb y zineb, Reacciona con, e inactiva, los grupos sulfhidrilo de aminoácidos y enzimas de células fúngicas, resultando en la interrupción del metabolismo lipídico , la respiración y la producción de trifosfato de adenosina
Antracnosis ***Colletrotrichum spp***	Benomilo: Metil 1 (butilcarbamoil) bencimidazol-2-il carbamato. Fungicida contra Antracnosis (*Colletotrichum gloeosporiodes*), Fumagina (*Capnodium spp*.), Mancha de la hoja (*Cercospora purpurea*) y Roña o sarna (*Sphaceloma perseae*). N-triclorometiltio-4-ciclohexeno-1,2-dicarboximida Fungicida contra: Mancha púrpura (*Alternaria porri*), Mancha foliar (*Cercospora capsici*) y Antracnosis (*Colletotrichum gloeosporiodes*).
Viruela ***Septoria lycopersici***	Azoxistrobina inhibición del proceso respiratorio de los hongos, impidiendo la germinación de las esporas y el desarrollo del patógeno
Cancro bacteriano ***Clavibacter michiganensis***	Estreptomicinina, derivado amino glucósido que realiza una inhibición de la síntesis proteica a nivel de la subunidad 30s del ribosoma.
Mancha bacteriana ***Xanthomonas axonopodis y Xanthomonas vesicatoria***	Oxicloruro de cobre, Mancozeb, Hidróxido y sulfato cúprico y Acbenzolar-5-metil, son sugeridos para manejar bacterias, sin embargo deben ser aplicados de modo preventivo, ya que estos actuarán como escudo, pero no entran a sanar a las plantas, por lo que precisan ser aplicados antes de que la enfermedad inicie.
Peca bacteriana ***Pseudomonas syringae***	Sulfato de Estreptomicina, Clorhidrato de Oxitetraciclina, Sulfato de Gentamicina Kasugamicina son antibióticos que actúan sobre las bacterias inhibiendo la síntesis de proteína a nivel de ribosoma. Debido a que estos dos antibióticos actúan a diferentes subunidades de ribosoma, se reducen las probabilidades de crear resistencia del patógeno y se obtiene un ataque más agresivo sobre la enfermedad.
Necrosis de la médula o tallo hueco ***Pseudomonas corrugata, P. mediterránea y P. viridiava***	
Marchitamiento bacteriano ***Ralstonia solanacearum***	
Podredumbre blanda del tallo ***Pectobacterium carotovorum***	
Peste negra ***tomato spotted wilt virus (TSWV), el Tomato chlorotic spot virus (TCSV), el Groundnut ring spot virus (GRSV) y el Impatiens necrotic spot virus (INSV*)**.	En el caso de virus que se transmiten por contacto, al realizar las labores Culturales desinfectar las manos y herramientas después de haber trabajado en una parcela afectada. Se puede usar agua con formol al 1 %, solución de fosfato trísódico al 10%, cloruro de lauryl, dimetil bencilamonio al 0,5%. There are currently no chemical options that are effective against either virus. Johnson, et al., 2015.
Mosaico ***Tomato mosaic virus (ToMV)***	
Virus de la cuchara ***Tomato vein streak virus (ToVSV) como la especie prevalente, la presencia del***	

Soybean blistering mosaic virus (SbBMV) y del Tomato Yellow spot virus (ToYSV).	

Los inconvenientes que presenta el control químico se han potenciado en los últimos años debido al cambio en los sistemas de cultivo (monocultivos, explotaciones intensivas, entre otras). Esto, unido a una mayor concienciación social, ante el deterioro medioambiental que supone la utilización masiva de compuestos químicos, ha provocado un gran interés en la búsqueda de sistemas de control alternativos (Serrano y Galindo, 2007). Así, en los últimos años, debido a las preocupaciones sobre la seguridad de los agentes antimicrobianos sintéticos, ha habido un aumento en el uso de las sustancias naturalmente desarrollados, que ha dado lugar a un enorme incremento en el uso de compuestos de origen natural como aceites esenciales y extractos vegetales de diversas partes de las plantas medicinales importantes como agentes antifúngicos (Hernández *et al*., 2007).

Antimicrobiano de origen natural

El método más importante para proteger a las plantas contra ataques microbianos es el uso de microbiocidas. Sin embargo, aunque existen muchos microbiocida en el mercado, la mayoría de ellos son tóxicos y tienen efectos indeseables para el hombre y el medio ambiente. Por su parte, los hidrocarburos hidrogenados como el bromuro de metilo tienen como consecuencia daño a la capa de ozono. Otros son no biodegradables y tienden a acumularse en el suelo, causando desequilibrio en el medioambiente. Es por eso que el desarrollo de fungicidas amigables con el medio ambiente y no dañino para la salud ha sido un campo importante de estudio (Mdee *et al*., 2009). Para la aplicación de los antimicrobianos de origen natural, se necesita comprobar su eficacia "*in- vitro*", en medios microbiológicos y en productos alimenticios. Las pruebas "*in -vitro*" proporcionan información valiosa acerca de la efectividad de un compuesto, como las variables que afectan a la actividad antimicrobiana, de la cual depende del tipo, género, especie y microorganismo a

probar (Rodríguez *et al*., 2011). Por ejemplo, las esporas bacterianas son más resistentes al efecto de los antimicrobianos que las células vegetales (Rodríguez *et al*., 2011). También el tipo de pared celular es un factor a considerar. Otra variable asociada a la efectividad de un agente antimicrobiano en los alimentos, es el número inicial de los microorganismos en el sistema. Por otro lado, Los agentes antimicrobianos de origen vegetal no contribuyen al desarrollo de cadenas de resistencia o alteran el ambiente del alimento de manera que crezcan otros organismos patógenos. Existe un número importante de reportes acerca de la actividad antimicrobiana de extractos, aceites, especias y condimentos, es difícil obtener estimaciones cuantitativas y hacer comparaciones de sus efectos debido, al menos parcialmente, a la gran variedad de métodos que se han utilizado para evaluar su efectividad (Rodríguez *et al*., 2011).

Plantas con actividad antimicrobiana

La búsqueda de estrategias, técnicas y métodos para incrementar la productividad agrícola y conserven el balance ecológico, sin exponer la salud humana, es un gran reto para la agricultura y su desarrollo (Gallegos *et al*., 2004). Existen antecedentes que indican el potencial antimicrobiano de componentes de especies vegetales distribuidas en la zona semidesértica de México. Entre estas especies vegetales se encuentra la gobernadora (*Larrea tridentata Cov*.) (Lira, 2003), la nuez (*Carya illinoensis*) y la granada (*Punica granatum*) se encuentran en regiones localizadas del semidesierto mexicano sin embargo poseen potencial, antibacteriano y antifúngico. Estos cultivos se caracterizan por tener una gran cantidad y variedad de polifenoles. Dentro de los compuestos polifenólicos que presentan la gobernadora (*Larrea tridentata Cov*.), la nuez (*Carya illinoensis*) y la granada (*Punica granatum*) están los galotaninos, elagitaninos, taninos complejos y taninos condensados, los cuales tienen funciones de protección contra mamíferos y microorganismos. Hay reportes de la inhibición del crecimiento bacteriano por extractos vegetales (Güven y *et al*., 2005, Güven y *et al*., 2006). Por otro lado se ha demostrado capacidad inhibitoria de extractos de plantas frente a bacterias que

afectan a la salud humana; por ejemplo *Staphylococcus aureus* fue inhibido con la aplicación de extracto de *Indigofera suffruticosa; Escherichia coli y Pseudomonas aerugeniosa* fueron inhibidos con extractos de *Lupinus angustifolius*; *Helicobacter pylori* fue inhibida con extractos de ajo. Los reportes de inhibición del crecimiento de bacterias fitopatógenas por extractos vegetales son menos numerosos, sin embargo representan una gran alternativa para el desarrollo de una nueva generación de bactericidas (Gallegos *et al*., 2004).

Métodos de obtención de extractos naturales a partir de plantas

Las plantas representan una fuente de comparación de diferentes grupos de compuestos antiespasmódicos, antisépticos, anticancerígenos, antimicrobianos, antifúngicos, entre otros; estas plantas han sido empleadas en su mayoría de forma empírica y cotidiana; sin embargo los investigadores han vuelto su mirada en la evaluación y caracterización de las plantas y sus constituyentes, por lo que la extracción de sus bioactivos representa una gran oportunidad y reto para los problemas que le atañen a la humanidad hoy en día (Tiwari *et al.,* 2011). Por extracción debemos entender como la separación de los componentes activos de la planta, a partir de los tejidos empleando de manera selectiva solventes mediante procedimientos estandarizados; así los productos obtenidos son una mezcla compleja de diversos metabolitos que pueden estar en estado líquido o semisólido, o bien se pueden encontrar liofilizados (Remington, 2011). Los métodos de extracción envuelven la separación de las fracciones de los compuestos activos de las plantas bajo estudio en componentes activos e inactivos empleando diferentes solventes, durante la etapa de extracción los solventes se difunden dentro del material de la planta y solubilizan los compuestos; una vez obtenidos los bioactivos estos pueden ser fraccionados y caracterizados por diferentes técnicas como cromatografía liquida de alta resolución acoplado a espectrometría de masas (HPLC-MS) o bien cromatografía por permeación en gel, capa fina o bien la cromatografía de papel, para su posterior empleo en el diseño de productos

industriales. Dentro de los bioactivos posibles se pueden encontrar metabolitos de la planta, alcaloides, terpenoides, flavonoides, ligananos y compuestos glicosídicos (Handa *et al.,* 2008). Las técnicas generales para la extracción de componentes bioactivos pueden ser la maceración, la infusión, la percolación, la digestión, la extracción continua (Soxhlet), la fermentación para extractos alcohólicos; la técnica de microondas asistida, la extracción por ultrasonido (sonicación); la técnica de extracción de fluidos supercríticos, la micro destilación, la maceración hidrolítica seguida de la destilación entre otros (Handa *et al.,* 2008). Los parámetros básicos que permiten conocer la calidad del extracto según Ncube *et al.*, (2008) corresponde a conocer la parte de la planta empleada en la extracción, así como los solventes empleados aunado a su metodología de extracción. El éxito de la determinación de compuestos bioactivos de un extracto depende de las propiedades del solvente, así como a su baja toxicidad, y su rápida absorción de manera fisiológica (Das *et al.*, 2010). En la Tabla 2 se muestran algunos de los así como los posibles compuestos bioactivos a obtener en la extracción de los diferentes tipos de solventes para el análisis de las plantas. Actualmente se han realizado estudios para el área agronómica de extractos vegetales metanólicos y acuosos con resultados efectivos para inhibir el crecimiento de diversos bacterias (Koduru *et al*., 2006). Extractos de *Cajanus cajon*, usando como solventes agua, éter de petróleo, etanol, cloroformo y metanol inhibieron el crecimiento de *E. coli, S. aureus* y *Salmonella typhimurum* (Okigbo *et al*., 2006). Por otro lado un procedimiento no estandarizado en la extracción puede provocar la degradación de los fitoquímicos presentes en las plantas, ocasionando a su vez variaciones en los resultados disminuyendo la reproducibilidad del experimento; por eso, los investigadores deben realizar esfuerzos para eliminar posibles fallas en la calidad de los extractos y así poder desarrollar la mejor manera de extracción de los componentes bioactivos de las plantas.

Tabla 2. Solventes empleados para la extracción de compuestos bioactivos.

Agua	Etanol	Metanol	Cloroformo	Éter	Acetona
Antocianinas	Taninos	Antocianinas	Terpenoides	Alcaloides	Fenol
Almidones	Polifenoles	Terpenoindes	Flavonoides	Terpenoides	Flavonoides
Taninos	Poliacetilenos	Saponinas		Cumarinas	
Saponinas	Flavonoides	Taninos		Ácidos grasos	
Terpenoides	Terpenoides	Xanthoxilenos			
Polipeptidos	Esteroles	Totarol			
Lectinas	Alcaloides	Lectonas			
		Flavonoides			
		Fenoles			
		Polifenoles			

Métodos de obtención de extractos naturales a partir de microorganismos

Dentro del campo de la obtención de extractos naturales se encuentra la obtención de metabolitos secundarios de microorganismos aplicados en control biológico, presentando ventajas sobre los métodos químicos frente a los daños al ecosistema y salud humana debido a que estos microorganismos (Bastas, 2014). Baker y Cook (1974) definen control biológico desde una perspectiva patológica en las plantas como "la reducción de la densidad de inoculo o actividades que produzcan la enfermedad de un patógeno o parásito en su estado activo o inactivo, por uno o más organismos, logrando de manera natural o mediante la manipulación del medio ambiente, huésped, o antagonista, por masa o introducción de uno o más antagonistas". De esta manera, el término "control biológico" o "biocontrol" se refiere al uso de una fuente natural de microorganismos los cuales son antagónicos a los patógenos que deseamos eliminar. El antagonismo entre microorganismos es un fenómeno que siempre ha estado presente en la naturaleza e involucra a hongos (incluyendo levaduras) y bacterias que habitan de manera natural en el suelo y en las superficies de los diferentes partes de las plantas (Wilson y Wisniewski, 1994).Uno de los enfoques del aislamiento de los microorganismos antagónicos para controlar las pérdidas es a través de la promoción del manejo de los

antagonistas naturales presentes en superficies de frutas y vegetales (Barkai, 2001). Dentro de los mecanismos involucrados en el biocontrol de antagonistas encontramos la antibiosis, producción de enzimas líticas, parasitismo, inducción de resistencia y competencia por nutrientes y espacio (Janisciewicz y Korsten, 2002). Entre los antagonistas estudiados en sistemas que involucran patógenos del suelo que causan secadera, pudriciones de raíz y de la corona, así como marchitez vascular, se involucra a *Trichoderma, Gliocladium, Pesudomonas, Bacillus, Pythium, Laestisaria,Sporidesmiun Coniothryium, Verticillum y Talaromyces*. Los hongos más utilizados para el control biológico de la secadera y pudriciones radicales son los Hyphomycetes y entre ellos los géneros *Trichoderma, Penicillum* y *Gliocladium.* (Casimiro, 2001). Utilizar agentes de control biológico, proporciona una mayor efectividad en el control de enfermedades de las plantas y para ello es necesario entender los sistemas de cultivo, la epidemiologia de la enfermedad, la biología y dinámica de la población de los antagonistas y la interacción entre todas ellas.

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Artículo I

REVIEW OF ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AND GROWTH-PROMOTING MICROORGANISM (GPM) AGAINST PHYTOPATHOGENIC BACTERIAL TOMATO CROP

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ABSTRACT

Tomato is the second vegetable more important crop in the world, but has problems with bacterial phytopathogen that causes economic losses. The effectiveness of bioalternatives for controlling phytopathogen bacterial tomato disease is importance nowadays. Scientists are working on the development of new techniques for bioalternatives to control bacterial tomato diseases trying to avoid the traditional chemical control, because side effects can affect human health and causes damage at environment. In this review summarizes the alternatives compounds of some extract plants, as well as some compounds by *Bacillus* used as plant-growth promoting rhizobacteria, and some compounds by *Trichoderma* like an plant-growth promoting fungi.

Key Words: Agrobiotechnology**,** Plant-extracts, Beneficial-Microorganism, Bacterial-phytopathogenic, Tomato.

INTRODUCTION

The past few decades agricultural sciences intensified her studies on get more food production by agrochemicals compounds, those one present as a relatively method of protection and safe crops (Compant *et al., 2005*). However one of the major problems in agriculture today are due to pathogenic microorganisms that affect de health plant, by this way the quantity and quality of the crop products. Nevertheless, increasing use of chemical

inputs causes several negative effects, development of pathogen resistance to the applied agents and their non-target environmental impacts (Sheikh *et al., 2013*). At the same time the increasing consumer demand for foods free or with low, if any added synthetic preservatives because could be toxic to human, forcing biotechnology agriculture to seek control sources against these microorganisms that are more friendly by environment and human(Agatemor, 2009). Several studies on the pathogen-toxic activities of plant secondary metabolites and beneficial microorganism have reported by bioalternatives control (Osorio *et al.,* 2010). These bioactive compounds are often active against a limited number of spices, including the specific targets, are biodegradable and potentially suitable for integrates use, they could be develop as a new classes of possibility safer diseases control agents (Soylu *et al.*, 2010).

Tomato (*Solanum lycopersicum*) is by far the most important vegetable crop; in terms of economic value, tomato constitutes 72% of the value of fresh vegetables produce worldwide (Hanssen *et al.*, 2010). With a worldwide production of 162 million metric tons and a value of over 37 billion international dollars in 2015 (Food and Agricultural Organization, United Nations, 2016). Number of biotic factors including viruses, bacteria, fungi and nematodes causing devastating diseases resulting in great economic losses (Arshad *et al.,* 2014) affects tomato production. Bacterial diseases are serious problem in the greenhouse and in open field production. The major pathogens are responsible for damage on tomato organs such as roots, stems, twigs, leaflets, leaf, buds, flowers and fruit in the war temperature regions of the world (Balestra *et al.,* 2009). These are *Pseudomonas syringae*, causal agent of bacterial speck; *Xanthomonas vesicatoria* causal agent of bacterial spot; *Clavivabter michiganensis* that causes bacterial canker, *Pseudomonas corrugate* agent causal of bacterial pith necrosis, *Ralstonia solanacearum* agent of bacterial built and *Agrobacterium tumefasensis* agent of crown gall disease (Yuliar *et al.,* 2015).

Actually have been report plant species with antifungal and antibacterial activities, mainly plant pathogens. For extraction of active phytochemicals against plant bacterial and fungal pathogens, the most commonly solvents used are methanol, ethanol, hexane, chloroform and diethyl ether (Mendez *et al.,* 2012). The use of the most of these solvents aren´t friendly by environment and aren't allowed in organic productions system, by this reason agrobiotechnology its focused on research on plants with high content polyphenols and organic solvents which are allowed to be used under organic production system (Castillo *et al*., 2010). The use of biologically based compounds in plant extracts its important because they constitute a rich source of phenols, flavonoids, quinons, tannin, alkaloids, saponins and sterols; some phytochemical of plant origin have been formulated as botanical pesticides and were considered successfully and friendly by environment and were integrated in pest management program (Soylu *et al.,* 2006).

In other hand agrobiotechnology, it has supported and focused in the development of use the beneficial microorganism on plant growth. Such as beneficial microorganisms referred as PGPR (plant-growth promoting rhizobacteria) or PGPF (plant growth promoting fungi) enhance plant growth trhoung numerous mechanism the protection of roots against infection by minor o major pathogens. (Gravel *et al.,* 2007). Rhizobacterias, defined as saprophytics bacterias that live in the plan rhizosphere and colonize the root system, as plant growth promoters. Colonization of the plant root system can lead reduced pathogen attack directly or competition for spaces, nutrients and ecological niches, and indirectly, through induction systemic resistance (Silva *et al*., 2003). The three families of *Bacillus* lipopeptides –

surfactins, iturins and fengycins- were at first mostly studied for their antagonism activity for a wide range of potential phythopathoges. The different structural traits and physicochemical properties of these effective surface- and membrane-active amphiphilic biomolecules explain their involvement in the most of the mechanism developed by bacteria for the biocontrol of different plant pathogens (Ongena & Jaques, 2007). By other way, some fungus like *Trichoderma* have economic importance for production of antibiotics an enzymes, degradation of xenobiotic compounds, biological control and inductions of systematic acquired resistance in plants by endophytism (Zhou *et al*., 2007; Brunner *et al.,* 2005). *Trichoderma* species can improve plant growth promotion, evidenced by increases in biomass, productivity, stress resistance and increase nutrient absorption. Presumed mechanism involved in the stimulation of plant growth, included interactions with roots, where *Trichoderma* penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain (Hoyos-Carvajal *et al.,* 2009). *Trichoderma* showed activity of glucanases, chithinases, cellulases and peroxidases evidence of the activation of the plan defense, also could produce metabolites with activities analogous to plant hormones (Harman, 2006).

The aim of this review consist on collect the currently results on investigation, by bio alternatives extracts and beneficial microorganism – *Bacillus* and *Trichoderma*- used by phytobacterial control on the tomato crop, to show the importance of using environmentally friendly technology.

Extracts Plants

Science ancient times, mankind has used plants to treat common diseases an some of these traditional medicines are still included as part of the habitual treatments of various maladies (Alviano & Alviano, 2009). The activity of plant extracts may therefore make possible in the actually the design of less expensive alternatives on different sciences as agrobiotechnology, to be used by friendly by environment, to generate a change on using chemical compounds (Castillo *et al*., 2010). Research on the use of plants is part of the ethnobotany, which has been define as the study of the interrelations between human groups and plants. For its interdisciplinary nature covers many areas including : botany, chemistry, medicine , pharmacology , toxicology , nutrition , agronomy , ecology , sociology , anthropology , linguistics, history and archeology , among others; allowing a wide range of approaches and applications (Bermúdez *et al.,* 2005). In Table 1, a compilation of the antibacterial shows properties of plant extracts used to control bacteria in different sciences areas in the world such as medicine, nutrition and agronomy. This in order to find alternatives that can be analyzed by agrobiotechnology to be efficient as antibacterial on tomato crop diseases and can be environmentally friendly with less secondary effects by health human. Research laboratories worldwide have found literally thousands of phytochemicals, which have in *in vitro* inhibitory effects on all types of microorganisms (Camacho-Corona *et al*., 2015). Actually scientific data are accumulating that demonstrate for many herbs and related essential oils healing properties useful in the prevention of diseases or in relieve their symptoms. Therefore, plant extracts in the form of decoction, infusion, tincture or essential oils represent an important bioalternative by the population for treatment of several diseases on different sciences (Sheikh *et al., 2013*). When infection or physical damages happen, many processes of the plant defense were activate. Some compounds were produce immediately, whereas phytoalexins are present only after two to three days (Alviano &

Alviano, 2009). Phenolic compounds substances generally have significant antimicrobial activity as well as flavonoids. Some phytochemicals actually showed the presence of effective biological compounds like alkaloids, amino acids, flavonoids, phenols, tannins, terpenoids, saponins and cumarines (Ibrahim & Sarhan, 2015). These derivate could be potential alternatives to the traditional chemical control of clinical pathogen and phytopathogenic bacteria. Furthermore, the development of natural antibacterial will help to decrease the negative effects of chemicals controls (Riviera *et al.,* 2014). Fractionation and characterization of these active compounds will be the future bioalternatives by agricultural sciences. However, of the future commercial exploitation of the plants found to show significant activities must take in to account not only biological properties, including acceptable levels of toxicity, but also the growing habits, ease of cultivation and availability of these plants to the local population(Körpe *et al.,* 2012).

Table 1. Plants in world that showed antibacterial activity.

Plant	Part used	Extract	Bioactive Compounds	Antibacterial activity	Reference
Allium stivum L.	Leaves	Hot and cold, water	ND.	*Ralstonia solanacearum*	Abo-Elyousr & Arnsan, 2006
Datura stramonium L.					
Nerium oleander L.					
Agave lechugilla	Leaves	Water, ethanol, lanolin and cocoa butter	Tannins, saponins and terpenes	*Enterobacter aerogenes, Escherichia coli, Salmonella typhi and Staphylococcus aureus*	Mendez *et al.*, 2012
Larrea Tridentata, tar bush F. *cernua*					
Lippia graveolen					
Yucca filifera					
Carya illinoensis	Husks on pecan nut				
Allium stivum L.	Clove	Water	Polyphenols, Allicin	*Pseudomonas syringae* pv. t*omato, Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *Michiganensis*	Belastra *et al.*, 2009
Ficus carica	Fruit				
Acccia nilotica	Leaves	Water	Alkaloids and Terpenoids	*Pseudomonas syringae and Xanthomonas axonnopodis*	Sheikh *et al.*, 2013
Catharanthus roseus					
Coleus aromaticus					
Plumbago zeylanica					
Santalum album					
Tinospora cardofolia					
Withania somnifera					

Propolis Turkey	ND	Methanol	ND	*Agrobacterium tumefaciens, Agrobacterium vitis, Clavibacter michiganensis* subsp.*michiganensis, Erwinia amylovora, Erwinia carotovora* pv. *Carotovora, Pseudomonas corrugata, Pseudomonas syringae* pv. p*haseolicola, syringae and tomato, Ralstonia solanacearum, Xhanthomonas campestris* pv. *campestris,and Xhantomonas axonopodis* pv. *vesicatoria*	Basim *et al.,* 2006
Pollen Turkey	ND				
Urtica dioica L.	Leaves, roots and seeds	Water and Methanol	Polyphenols	*Escherichia coli, Enterococcus gallinarum and fecalis, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aureginosa, Shigella spp, Bacillus subtilis, Clavibacter michiganensis* subsp. *Michiganensis, Pseudomona syryngae* pv. t*omato, Xhantomonas axonopodis* pv. *vesicatoria, Erwinia carotovora and amylovora*	Körpe *et al.,* 2012
Urticua pilulifera L.					
Azadirachta indica A. *Juss*	ND	Essential oil	ND	*Clavibacter michiganensis subsp. michiganensis*	Borboa-Flores *et al.,* 2010
Brassica nigra					
Buxus chinensis					
Capsicum annuum					
Chaix mentha piperita L.					

Cinnamomun zeylanicum					
Citrus aurotinon L.					
Eucalyptus globulus Labill					
Lavandula officinalis					
Lippia palmeri Watson					
Medaleluca viridiflora Gaerin					
Melaluca alternifolia					
Origanum Vulgare L.					
Petroselinum sativum					
Ricinus comunis					
Rosmarinus officinalisL.					
Salvia Officinalis L.					
Thymus vulgaris L.					
Trigonella foenum graecum L.					
Lavandula angustifolia	Complete plant	Essential oil	Cromatography-mass spectometry (GC-MS), alpha-pinene, camphene, beta-myrcene, alpha terpinene, p-cymene, beta-phellandrene,	*Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia amylovora* and *Xhantomonas campestris*	Daferera *et al.*, 2003
Mentha pulegium					
Origanum dictamnus					
Origanum majorana					
Origanum Vulgare					
Rosmarinus officinalis					
Salvia fruticosa					

Thymus capitatus			eucalyptol, terpinene, terpinolene, linalool, thujone, camphor, nerol, thymol, carvacol, neryl acetate		
Lippia origanoides	leaves	Petroleum ether, hexane and ethanol	Flavanoids, Tannins, steroids, alkaloids, carotenoids	*Escherichia coli, Pseudomonas aeruginosa, Aeroma hydrophilia, Proteus mirabilis, Enterobacter clocae, Klebsiella Pneumoniae, Staphylococcus aureus and Enteroccoccus galliarum*	Henao *et al.*, 2009
Ocimum basilicum L.	Leaves	Ethanol	Alkaloids,Flavanoids, Saponins, Tannins, Terpenoids and Steroids	*Escherichia coli*	Ibrahim & Sarhan, 2015
Larrea tridentata, tar bush F. cernua	Leaves and stems	70 % Ethanol	Tannins	*Enterobacter aerogenes, Escherichia coli, Salmonella typhi and Staphylococcus aureus*	Riviera *et al.*, 2014
Opuntia ficus-indicata	Paddle cactus				
Calycopteris floribunda	Flowers	Petroleum ether, chloroform and methanol	Phenols and Flavonoids	*Staphylococcus aerus , Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli*	Pavithra *et al.*, 2013
Humboldtia brunonis					
Kydia calycina					
Acacia farnesiana	Flowers and leaves	Methanol	Flavanoids and quinones	*Klebsiella pneumoniae, Staphylococcus aureus,*	Menchaca *et al.*, 2013

Euphorbia antisiphylitica	Leaves			*Escherichia coli, Enterobacter aerogenes, Enterobacter Clocae*	
Fouquieriaceae splendens					
Leucophyllum frutescens					
Tecoma stans					
Larrea Tridentata	Leaves	Methanol, hexane, dicloromethane, ethyl acetate, ethanol	Dihydroguaiaretic acid (NDGA), Kaempferol and quercitin	*Staphylococcus saprhophyticus and epidermidis, Enterococcus faecalis,Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli*	Martins *et al.,* 2013
Ambrosia ambrosioides	Aerial parts, fruits and Flowers	Methanol, clhoroform, dichloromethane and ethyl acetate	ND	*Mycobacterium tuberculosis*	Robles-Zepeda *et al.,* 2013
Ambrosia confertiflora					
Guaiacum coulteri					
Backousia citriodora	Plant Mixture	Water, ethanol and peptide	Phenols	*Staphylococcus aureus, Escherichia Coli, Bacillus cereus and Pseudomonas aeruginosa*	Shami *et al.,* 2013
Citru australasica					
Lophopyrim ponticum					
Terminalia ferdinandiana					
Aristolochia cymbifera	Stem	Water and ethanol	ND	*Prevotella intermedia, Phorphyromonas gingivalis, Fusobacterium nucleatum, Streptoccoccus mutans*	Aliviano *et al.*, 2008
Caesalpinia pyramidalis	Leaves				
Cocos nucifera	Husk fiber				
Ziziphus joazeir	Innerbark				

Artemisia nilagarica	Leaves	Chloroform, diethyl ether, ethanol, hexane, methanol and petrolumm ether	Alkaloids, Flavonoids, Phenols, Tannins, Quinines, Saponins and terpenoids	*Erwinia sp. Xhanthomonas campestris, Pseudomonas syringae, Clavibacter michiganensis, Yersenia enterolitica, Klebsiella pneumoniae, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Shigella falxneri, Bacillus subtillis, Enterococcus faecalis, Staphyloccoccus aureus.*	Ahameethunisa & Hopper, 2010
Alhagi maurorum	Leaves	Ethanol	ND	*Escherichia coli, Moraxella Lacunata, Proteus merabiles and vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtillis, Mircococcus Kirstiniae, Micrococcus luteus, Sarcina ventricull, Staphylococcus aureus, haemolitucs and byogenes*	Zain *et al.*, 2012
Chenopodium murale					
Convolvulus fatmensis					
Conyza dioscoridis					
Cynanchum acutum					
Diplotaxis acris					
Euphorbia cunaeata					
Origanum syriacum					
Solenostemma argel					
Tamarix aphylla					
Allium stivum L.	Complete plant	Ethanol	Saponin, tannin, scopolamine, atropine, allicin, flavonoids, chrysin, liquiritigenin, naringenin, kaempferol and quercitin	*Vibrio spp.*	Sharma & Patel, 2009
Datura stramonium L.					
Sara indica					

Lonicera alpigena	Complete plant	Ethanol	ND	*Staphylococcus aureus*	Quave *et al.*, 2008
Castanea sativa					
Juglans regia					
Ballota nigra					
Rosmarinusofficinalis					
Leopoldia comosa					
Malva sylvestris					
Cyclamen hederifolium					
Rosa canica					
Rubus ulminifolius					
Crataegus mexicana	Leaves and Fruits	Water, hexane, ethanol	ND	*Stenotrophomonas maltophilia, Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Klesiella Pneumoniae, Streptoccocus aureus, Listeria monocytogenes, Enterocccus faecalis and Mycobacterium tuberculosis*	Camacho-Corona *et al.*, 2015
Hyptis albida					
Ocimum baislicum					
Prunucs serotina					
Melia azederach L.	Leaves	Ethanol	ND	*Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Enterobcoccus faecalis and Burkhordelia glumae*	Sierra *et al.*, 2012

*Not determinate (ND) by reference author.

Bacillus as Plant-growth promoting bacteria (PGPB)

The population dynamic of the human has led to the exploitation of natural resources in search of a way to meet the nutritional needs of the billions of people inhibiting the planet. This need has led to the uses of high-efficiency chemical materials in agriculture; strategies used in modern agriculture have negative environmental impact that human yet fully understand (Castillo *et al.,* 2010). As an alternative to the use of chemical compounds, the use of rhizopheric bacteria has been proposed as plant growth promoting bacteria (PGPB); these bacteria can stimulate de growth directly or indirectly and show several mechanism that interact with each other to establish beneficial relationships, especially with the roots of target plants (Camelo *et al.,* 2011). Between direct mechanisms, it´s found the biological nitrogen fixation (BNF), production of hormones plants (PHP), and biosolubilization by phosphate (BP). In other way indirect mechanisms covers biocontrol mechanism by antagonism (BMA), which can be by presence of antibiotics, siderophore, auxins, lytic enzymes and antifungal metabolites, others indirect mechanism can be by controllers stress, by regulation of ethylene levels in plant (CS), induced systemic resistance (ISR) or volatile organic compounds (VOCs) (Molina-Romero *et al.,* 2015). Representatives of many different bacterial genera have been introduce into planting materials to improve growth crop. These bacterial genera *Acinetobacter, Agrobacterium, Arthrobacter, Azopirillum, Bacillus, Bradyrhizobium, Frankia, Pseudomonas, Rhizobium, Serratia, Streptomyces and Thiobacillus,* and many other has been reported with rhizobacteria growth promoting activity in tomato crops (Alfonso *et al.,* 2005; Haas & Défago, 2005, Kloepper *et al.,* 2004; Nihorimbere *et al.,* 2010).

A number of *Bacillus* strains express activities that suppress necrotizing pathogens/parasites or otherwise promote plant growth (Choudhary & Johri, 2009). The most studied of the insect pathogens are those classified as *Bacillus thuringiensis* which is distinguish from the common saprophytic species. Several species of *Bacillus* produce toxins that are inhibitory growth or activities fungal, bacterial and nematode pathogens of plants, wherein most thoroughly studied species include *B. subtilis* (Pinchuk *et al.,* 2002). In addition, a number of studies reported direct antagonism by several species that include *B. amyloliquefaciens, B. cereus, B. licheniformis, B. megaterium,* and *B, mycoides* and *B. pumilis* (Choudhary and Johri, 2009). In Table 2, examples of these and others bacteria's are show with their action against phytopathogen of some plants. *B. subtilis* strains have been develop commercially as a formulation and testes against several crop diseases (Latha *et al.,*2009), cells by these are capable of forming dormant spores that are resistance to extreme conditions and thus can be easily formulated and store (Chen *et al.,* 2013). *B. subtilis* produce, catabolic enzymes like proteases, chitinases and glucanases, produces peptide antibiotics and small molecules to contribute to pathogen suppression. Some lipopeptide antibiotics of *B. subtilis* can be iturin A and surfactin that could suppress diseases on tomato whereas zwittermicin A from *B. cereous* hove correlated to suppression disease in alfalfa (Ramamoorthy *et al.,* 2001 & Kloepper *et al.,* 2004).

The action mode of PGPB is not always the same, depends on the plant and crop with which they are interacting. PGPR is a multigene process influenced by some factors, therefore understanding of these processes and elucidate the mechanism of control tomato phytopathogenic bacteria need continue developing knowledge by scientific and agrobiotechnology sciences (Srivastava *et al.,* 2012).

Table 2. Bacterial biocontrol in different plants by beneficial bacteria's.

Bacterial Strain	Plant species	Pathogen	Mechanisms, activity or elicitors	References
P. aeruginosa 7NSK2	Tomato	*Botrytis cinerea*	Pyochelin and Pyocyanin	Audenaert *et al., 2002*
P. fluorescens CHAO	Tomato	*Meloidogune javanica*	2,4 DAPG (siacetypholoroglucinol)	Siddiqui & Sakuat, 2004
P. fluorescens Q2-87	Arabidopsis	*Pseudomonas syringae* pv. *Tomato*	2,4 DAPG (siacetypholoroglucinol)	Weller *et al.,* 2004
P. fluorescens GRP3	Tomato	*Pseudomonas syringae*	Siderophores	Meziane *et al., 2005*
Bacillus sp. BB11	Tomato	*Ralstonia calanacearum*	Biological control	Jian-Hua *et al.,* 2004
P. aureofaciens, corrugata and aphanidermatun	Cucumber	*Pythium aphanidermatum*	Stimulated activity enzymatic of phenylalanine ammonia-lyase (PAL), perxidase (PO), and polyphenol oxidase (PPO)	Chen *et al.*,2000
B. subtilis CBR05	Tomato	*Xanthomonas campestris* pv. *Vesicatoria*	Stimulated activity enzymatic of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase.	Chandrasekaran & Chun, 2016
B. hunamae MTI-641	Tomato	*Burkholderia unamaer*	Biological nitrogen fixing and enzymatic activity by 1-aminoacilclopropan-1-coboxylate desaminase	Caballero-Mellado *et al.*, 2004
B. amyloliquefaciens S13-3	Tomato	*Ralstonia solanacearum and Oidium neolycopersici*	Antibiotic production	Yamamoto *et al.,* 2015
A. zospirillum brasilense	Tomato	*Clavibacter michiganensis subsp. Michiganensis*	Antibiotic production	Romero *et al.,* 2014

B. Subtilis	Tomato	*Clavibacter michiganensis subsp. Michiganensis*	Bilogical control	Rojas, 2014
B. thuringiensis BUPM103	Tomato	*Agrobacterium tumefaciens*	Bacteriocin	Kamoun *et al.*, 2011
B. methylotrophicus 39b	Tomato	*Agrobacterium tumefaciens*	Lipopeptides as surfactins, iturins and fengycins	Frikha-Gargouri *et al.*, 2017
B. amyloliquefaciens SQR-9	Tomato	*Ralstonia solanacearum*	Volatile organic compounds (VOCs)	Raza *et al.*, 2016
Streptomyces spp.	Tomato	*Clavibacter michiganensis subsp. Michiganensis*	Biological control	Zhang *et al.*, 2010
R. aquatilis	Tomato	*Xanthomonas campestris pv. vesicatoria*	Biological control	El-Hendawy *et al.*, 2005
B. amyloliquefaciens and pumilus	Tomato	*Xanthomonas campestris pv. Vesicatoria*	Stimulated activity enzymatic of, perxidase (PO), and polyphenol oxidase (PPO)	Lanna-Filho *et al.*, 2013
B. subtilis QST 713	Tomato	*Pseudomonas syrigae pv. Tomato*	induced systemic resistance (ISR) genes PR1a, PR1b and Pin 2	Fousia *et al.*, 2016
B. subtilis, amyloliquefaciens, cereus, and pumilus	Tomato	*Fusarium solani*	Phosphate solubilization, Hydrogen cyanide (HCN) production, Indole acetic acid (IAA) production	Ajilogba *et al.*, 2013
B. megaterium MB3, subtilis MB14, subtilis MB99 and Amyloliquefaciens	Tomato	*Rhizoctonia solani*	Able to produce chitinase, B-1,3-Glucanase and protease	Manoj *et al.*, 2012

Trichoderma as plant growth promoting fungi (PGPF)

Another alternative to control phytopathogen bacteria's in tomato in the biological control as plant growth-promoting agent is *Trichoderma* spp. These agent is the most studied for their effects on reducing plant diseases (Avis *et al.,* 2008). *Trichoderma* (teleomorph Hypocrea) is a hemibiotitrophic fungus effective in reducing the severity of plant diseases trough several mechanisms, such as antagonism and mycoparasitism attacking or inhibiting the growth of plant pathogens directly or by inducing systemic and localize resistance plants (Fontanelle *et al.,* 2011). The fungal genus *Trichoderma* includes species of economic importance for production of antibiotics and enzymes, degradation of xenobiotic compounds, biological control activity against fungi, bacteria's and nematodes, and induction of systematic acquired resistance in plants by endophytism (Hoyos- Carvajal *et al.,* 2009). *Trichoderma* are widely used as biofertilizer and biopesticide in commercial formulates because of the multiple beneficial effects on plant growth a diseases resistance. (Tucci *et al.,* 2011). The genus *Trichoderma* produce numerous bioactive secondary metabolites which are species and strains dependent, including volatile and nonvolatile antifungal substances, some rhizophere-competent strains that can colonize root surfaces have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer efficiency utilization, percentage and rate of seed germination (Gal-Hemed *et al.*, 2011.) However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in- vitro* and *in-vivo* conditions to control diseases. Therefore, specific isolates are needed for successful control of particular pathogen, in Table 3 shows examples of *Trichodermas* as a biocontrol agent against different bacterial pathogens crops. The most common biological control agent of the *Trichoderma* genus strains are *Trichoderma harzianum, Trichoderma virens and Trichoderma viride* (Xiao-Yan *et al.,* 2006).

Table 3. Different *Trichoderma* strains against bacterial pathogens

***Trichoderma* Strain**	**Plant**	**Pathogen**	**Mechanisms, activity or elicitors**	**References**
T. harzianum	Tomato	*Clavibacter michiganensis subsp. michiganensis*	Lysosime and prevent activity	Utkhede & Koch, 2004
T. asperelloides T203	*Tomato*	*Pseudomonas syringae* pv. *tomate*	Increase level of WRKY 40 trascription factors and lipid transfer protein (LTP4)	Brotman *et al.*, 2012
T. atroviride and virens	*Tomato*	*Alternaria solani* and *Pseudomonas syringae* pv. *tomate*	Secret protein Sm1 and Epl1, wich elicital local and sistemic disease resistance.	Salas-Marina *et al.*, 2015
T. harzianum and asperellum	*Tomato*	*Xhantomonas campestris pv. vesicatoria*	Inducing acquired systemic disease resistance by chitinolytic and b-1,3-glucanolytic activitie	Saksirirat *et al., 2009*
T. harzianum	*Tomato*	*Xhantomonas campestris pv. vesicatoria*	Biocontrol activity, antagonism dosage	Suárez-Estrella *et al.*,2014
T. harzianum	*Tomato*	*Ralstonia Solanacearum*	Biocontrol activity	Liza & Bora *et al;* 2009
Trichoderma spp.	*Tomato*	*Xhantomonas euvesicatoria*, and *Alternaria solani*	Antagonistic activity, are able to degrade cellulose	Fontenelle *et al.*, 2011
T. harzianum T23	*Activity in-vitro*	*Clavibacter michiganensis and Erwinia Amylovora*	Production of viridiofungin A (VFA)	El-Hasan *et al.*, 2009
T. reesei	*Arabidopsis and tomato*	*Clavibacter michiganensis*	Production extracellular ezyme swollenin	Saloheimo *et al*., 2002
Trichoderma spp.	*Rice*	*Xhantomonas oryzae* pv. *Oryzae*	Biocontrol activity	Gokil-Prasad & Sinha, 2012

T. harzianum	*Cotton*	*Xhantomonas campestris pv. Malvacearum*	Induction of Systemic resistance by activity of peroxidase, phenylalanine ammonialyase, polyphenol oxidase an b-1,3-glucanase	Raghavendra *et al.*, 2013
T. asperellum T-203	*Cucumber*	*Pseudomonas syringae pv. Lachrymans*	Induction of sytemic resistance and accumulation of phytoalexins	Yedida *et al.*, 2003
T.harzianum	Soil, vermicompost	*Ralstonia Solanacearum and Meloidoyne incognita*	Biocontrol activity	Liza & Bora *et al.*, 2009
T. harzianum and viride	*Potato*	*Erwinia carotovora subsp. Carotovora*	Antagonistic effect	Sandipan *et al.*,2015
T. virens PS1-7	*Rice*	*Burkholderia plantarii*	produced carot4-en-9,10-diol a sesquiterpene-type autoregulatory signal molecule	Wang *et al., 2013*
T.asperelloides	*Cucumber*	*Pseudomonas syringae*	Protein swollenin, local defense	Brotman *et al*., 2008
T.virens	*Cucumber*	*Pseudomonas syringae*	Elicitation of systemic defences by 18 mer peptaibols	Viterbo *et al ., 2008*
T. harzianum and viride	*Banano*	*Ralstonia solanacearum race 2*	Biocontrol activity	Ceballos *et al., 2014*
T. asperellum T34	*Arabidopsis*	*Pseudomonas syringae* pv. *tomato*	MYB72, a node of convergence in induced sytemic resistance triggered	Segarra *et al.*, 2009
T. Koningii SMF2	*in vitro*	*Ralstonia solanacearum, Erwinia carotovora*pv. *Catotovora* and *Clavibacter michiganensis* ssp. *michiganensis*	Antimicrobial metabolits (Trichokonins)	Xia-Yan *et al.*, 2006

CONCLUSION

The need for increasing agricultural productivity and quality has led to an excessive use of chemical fertilizer, creating serius environmental pollution. The use of biofertilizers an biopesticides is an alternative for sustaining high production with low ecological Impact. This review records several results about the use of extract plants, plan growth promoting rhizobacteria and fungi (with potential bioactive properties that exhibited significant antimicrobial, antioxidant activity and growth activity) that support their use in the treatment of some phytopathogen tomato diseases. The Table 1, 2, and 3 shows the summary of some bioalternatives that can be use by agricultural science. Attention to this issue could usher in badly needed new area of bioalternative control treatment on bacterial tomato crop diseases by using friendly alternatives with environment. Finally, additional tests, including development of experimental models evaluating the agricultural applicability, are required before considering these options real promising compounds.

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Artículo II

Antibacterial activity *in vitro* of *Lippia graveolens* and *Origanum vulgare* against phytopathogen bacteria

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Abstract

Agrobaterium tumefacensis, Clavibacter michiganensis subsp. *michiganensis*, *Peudomonas syringae, Rasltonia solanacearum*, *and Xanthomonas axonopodis,* are considered some of the most destructive bacterial diseases of tomato crops. Currently, the use of friendly products to environments a tendency to avoid secondary effects by human health, and generates a new search for biological compounds to control diseases in crops. *Origanum vulgare* and *Lippia graveolens* are plants present in the majority of Mexican territory. The genus *Lippia* and *Origanum* have also been shown to have antimicrobial and antifungal activity for its high content of phenolic compounds (flavonoids), in different regions such as South and Central America, tropical Africa and Mediterranean. The aim of this study was to evaluate the antibacterial activity of *Lippia graveolens* and *Origanum vulgare* extracts on *Agrobaterium tumefacensis, Clavibacter michiganensis* subsp. *michiganensis*, *Peudomonas syringae, Rasltonia solanacearum*, *and Xanthomonas axonopodis*. The extracts of *L. graveolens* showed the best IC_{50} =19.01µg/mL, 12.71 µg/mL and 2.03 µg/mL by *A. tumefacensis, C. michiganensis* and *P. syringae,* respectively. Moreover, by *O. vulgare* exhibited the best IC_{50} against *P. syringae* and *R. solanacearum.* The extracts showed high phenolic and flavonoid content, as well as high antioxidant activity, and antibacterial activity.

Key words: Antibacterial activity, *Origanum vulgare*, *Lippia graveolens*, antioxidant activity, phenolic content.

1. Introduction

Tomatoes are among the highest-value crops in the world. In the United States-the world's second largest grower behind China-they account for more than a billion dollars in sales annually. Nutritionally, they are important sources of vitamins A and C (Price, 2017). Tomato (*Solanum lycopersicum* L.) crop is of great importance in Mexico (Jasso de Rodriguez et al., 2015). The country produces 3% of the world's production and is the second global exporter of this vegetable after Netherlands (SAGARPA, 2010). Bacterial diseases of tomatoes are one of the most serious and destructive diseases, affecting the plant development in greenhouse and field. Under field conditions, bacteria can cause localized epidemics affecting young developing fruit; in greenhouse, total crop losses can occur, when conditions are optimal, in other hand losses in yield can be up to 60% in some tomato fields (LeBoeufet al., 2016).*Clavibacter michiganensis* subsp. *michiganensis*, causal agent of tomato bacterial canker, is a seed borne pathogen and is considered one of the most destructive bacterial diseases of this crop. For this reason, in several countries is a quarantine pathogen (León et al*.,* 2011). Some symptoms caused by *C. michiganensis* subsp. *michiganensis* are wilt vascular discoloration, cankers formed on stems, petioles, marginal necrosis of leaflets which appear dried, and curl upward, "Bird´s- eye spots" develop on fruit and small white blister-like spots on stems (Borboaet al*.,* 2009). On the other hand, bacterial wilt caused by *Ralstonia solanacearum* race 4 is serious soilborn disease in the production of tomatoes in regions with tropical, subtropical and warm climates (Kimet al., 2016). This bacteria infects plants through roots and colonizing stem vascular tissue, the initial symptom in mature plants is wilting of upper leaves during hot days followed by recovery throughout the evening and early hours of the morning (Pradhananget al*.,* 2003). In addition, bacterial speck caused by *Pseudomonas syringae* pv. *tomato*, is considered one of the major diseases in many countries, , dark lesions present in infected plant parts are cause of depreciation of fruit quality and economical losses(Da-Silva et al.,2014). Bacterial spot of tomato is

a disease that affects foliage and fruit, is caused by *Xanthomonas campestris* pv. vesicatoria, fruits of tomato show superficial corky spots or scabs, and this bacteria also can cause canker-like splits in stems (Lee et al., 2012). The *Agrobacterium* genus belongs to the Rhizobiaceae family of alpha-Proteobacteria includes plant pathogens that cause crown gall and hairy root diseases (Slater et al., 2009). The soil borne bacterium *Agrobacterium tumefacensis* induces tumors in more than 1000 different dicotyledonous and some monocotyledonous plant species, including tomato crop; the pathogenicity strains results from the presence of tumor-inducing (Ti) plasmids (Toklikishvili et al., 2010).

Chemical bactericides have been widely used in disease control on tomato crop and are not easily biodegradable (Jasso de Rodríguez et al., 2011), they tend to persist in the environment, and have affected people´s health through residual toxic compounds present in the food for human consumption (Palacios-Nava and Moreno-Tetlacuilo, 2004).Nevertheless, the phytopathogens have developed resistance to these bactericides (Rivas *et al*., 2005). Research focused on plant-derived bactericides and their possible applications in agriculture have been intensified (Rojas-Fernández et al., 2008). These products have been suggested as effective substitutes of chemical pesticides (Bajpaiet al*.,* 2010). Thebactericidal effect have been attributed to the antioxidant compounds which give antimicrobial activity to the plants (Zengin and Baysal, 2014).*L. graveolens* and *O. vulgare* are active against bacteria, yeasts, and molds, in which thymol and carvacrol are mainly responsible for these properties (Gutierrez et al., 2009; Rivero-Cruz et al., 2011; Muriel-Galet et al., 2012). These compounds disrupt the cell membrane, causing an increased permeability/disintegration. Thymol can up- or downregulate genes involved in outer membrane protein synthesis (Radulović et al., 2013). Nevertheless, antimicrobial activity could be influenced by the composition and bioactive molecules as affected by geographical origin, variety, growth conditions, seasonal variations, vegetative cycle, environmental and soil factors, storage time, and leaves drying method (Novak et

al., 2002; Tekwu et al, 2012). There are more than 40 different classes of herbs known as oregano (Huerta, 1997).The objective of this study was to evaluate the antibacterial activity *in-vitro* of extracts from *Origanum vulgare* and *Lippia graveolens*, against *A*. *tumefacensis, C. michiganensis*, *P. syringae, R. solanacearum*, *and X. axonopodis* for identify a natural alternative to control phytopathogen bacteria.

2. Material and methods

2.1. Plant collection

Stems with leaves of *O.vulgare* were collected in nearby areas of Tapachula, Chiapas, Mexico (14°59'14.8"N and 92°15'55.4"W) and *L. graveolens* (stems with leaves) were collected in the municipality of General Cepeda, Coahuila, Mexico (25°22´42.2" N and 101°27´53.1" W). both of them were collected during summer season (June, 2016). The samples were labeled and transported in brown paper bags inside iceboxes, the General Cepeda samples were weighed the same day of the collection and the samples of oregano took two days, to arrive at the Phytopathology Laboratory from Parasitology Department at the Universidad Autónoma Agraria Antonio Narro (UAAAN).Immediately, leaves and stems were separated. Vegetal tissue was dehydrated at room temperature for 7-10 days, after was dehydrated using conventional oven Quincy lab, Chicago, USA Inc. 20GCE-LT at 60°C for 3 days at constant weight, and ground using a miller CUISINART, Supreme Grind ^{Tm} Automatic Burr Mill Demo NJ,USA model DBM-8 using a 1 mm screen. The powder was stored in dark bottles at room temperature until extraction was performed (Castillo et al., 2010).

2.2. Plant extraction

In order to obtain the plant extract, the method described by Shami et al., 2013; was followed with some modifications. Briefly, fourteen g samples of homogenized dried powder of grounded leaves and others with grounded stems, were added to 200 mL of absolute ethanol or water (Jasso de Rodriguez et al., 2015); and stirred in a stirring plate (Thermo Scientific CIMAREC model SP131325Q, USA) at room temperature for 3 days with the aid of a magnetic stirrer, in darkness. Then, the mixture was filtered through Whatman No. 1 filter paper. The extracts of both plant species were separated in two equal parts, one of them was stored in Eppendorf tubes (*Lippia graveolens* leaf H_2O crude(Lg.L.H.C.), *Lippia graveolens* stem H_2O crude (Lg.S.H.C.), *Lippia graveolens* leaf EtOH crude (Lg.L.E.C.) ,*Lippia graveolens* stem EtOH crude(Lg.S.E.C.) and *Origanum vulgare* leaf H_2O crude (Ov.L.H.C.), *Origanum vulgare* stem H_2O crude (Ov.S.H.C.), *Origanum vulgare* leaf EtOH crude (Ov.L.E.C), *Origanum vulgare* stem EtOH crude(Ov.S.E.C.)) and placed in a freezer at -10°C until its use in the bioassays. To the other half (*Lippia graveolens* leaf H_2O powder (Lg.L.H.P.), *Lippia graveolens* stem H_2O powder (Lg.S.H.P.), *Lippia graveolens* leaf EtOH powder (Lg.L.E.P.), *Lippia graveolens* stem EtOH powder (Lg.S.E.P), and*Origanum vulgare* leaf H_2O powder (Ov.L.H.P.), *Origanum vulgare* stem H_2O powder (Ov.S.H.P.), *Origanum vulgare* leaf EtOH powder (Ov.L.E.P.), *Origanum vulgare* stem EtOH powder (Ov.S.E.P.)), the solvent was removed by rotary evaporation (IKA-RV 10 digital V, USA Inc.) under reduced pressure at temperatures below 60°C (Martins et al., 2014), the remaining solvent was eliminated by placing the flask on drying oven at constant weight. The extract was preserved in Eppendorf tubes and placed in a freezer at -10°C until it's used in the bioassays.

2.3. Mineral content of extracts

The aqueous and ethanol extracts of *L. graveolens* and *O.vulgare* were analyzed by Fe, K, Na and Ca, using methods reported by Association of Official Analytical Chemists (2014). About 0.5 g of sample was put into a burning cup and 15 ml HNO_3 were added. The sample was incinerated in oven Quincy

lab, Chicago, USA Inc. 20GCE-LT at 200°C and dissolved ash was diluted to a certain volume with water. Concentrations were determinated with an ICP-AES Varian Vista, USA; with the same conditions by Ozcan et al., 2004 was followed. The results were expressed on dry weight basis (mg/100 g per plant of used portion of dried weight).

2.4 *Phytochemical analysis of extracts*

2.4.1 *Phytochemical screening*

The water and ethanol extracts were used for preliminary qualitative screening of phytochemicals such as alkaloids (Dragendorff and Sonneschain tests) carbohydrates (Molisch´s test), carotenoids (H_2SO_4 and $FeCl_3$) , coumarines (Erlich test), flavonoids (Shinoda and NaOH tests),free reducing sugar (Fehling´s and Benedict tests), glycosides (Gringnard), polysaccharides (lugol test),purines (HCl test), quinones (NH_4OH, H_2SO_4 and Börntraguer test), saponins (foam test), sterols andterpens (Liberman-Burchard and Rhosenthalertest),soluble starch (KOH and H_2SO_4), tannins ($FeCl_3$, $K_3[Fe(CN)_6]$ and gelatin tests), (Sahgalet al.,2009; Usman et al., 2009)

2.4.2. Determination of total phenols content (TPC)

The TPC of water and ethanol extracts of *L. graveolens* and *O.vulgare* were evaluated with Folin-Ciocalteu reagent, following the IFC method (Commission Regulation, EEC No 2676/90).Briefly, 20 µL of extracts, blank and calibration curve((0.97, 1.9, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000, 2000, 3000, mg/L, r^2=0.9833) were mixed with 120 µL of NA_2CO_3 (15% w/v), 30 µL of Folin-Ciocalteu reagent and 400 µL of water. The reaction was performed at 50°C for 5 min and absorbance was read at 700 nm. The standard used was gallic acid. Total phenols content was expressed as milligram gallic acid equivalent (mgGAE)/g dry weight plant material.

2.4.3. Determination of total flavonoids content (TFC)

The TFC content was performed by the chloride aluminum method as described by Chan et al. (2002). The absorbance was read at 510 nm using a spectrophotometer (Thermo Scientific™ Multiskan™ GO, USA).The standard used was quercetin. A calibration curve was prepared using a standard solution of quercetin (calibration curve (0.97, 1.9, 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000, 2000, 3000, mg/L, r^2=0.9899). Total flavonoids content was expressed as milligram quercetin (mg of QE)/ g dry weight plant material.

2.4.4. Antioxidant capacity of extracts (DPPH)

The antioxidant activity of extracts was carried out by the1, 1-diphenyl-2-picrylhydrazyl (DPPH●) assay according (Martins et al., 2012). A solution of DPPH● (60 µM) reagent was prepared, then, 2950 µL of this solution were added to 50 µL of sample extract. The mixture was vigorously shaken and then incubated for 30 min in the dark at room temperature by continuous monitoring of the decrease of absorption at 517 nm. The control solution contained 100 µL of distilled water. The radical scavenging activity was expressed as the inhibition of percentage by following equation:

% Inhibition of DPPH = (1-As/Ac) *100

Where A_c and A_s are the absorbance of the control solution and the absorbance of the sample solutions, respectively.

2.5 Antibacterial activity

2.5.1. Isolation of bacterial strains

Bacterias (*Agrobaterium tumefacensis, Clavibacter michiganensis* subsp. *michiganensis*, *Peudomonas syringae, Rasltonia solanacearum*, *and Xanthomonas axonopodis*) were isolated from the roots, leaves and stems of tomato plants that presented the symptoms of the diseases. The sampling was carried out in production plots established in: San Roberto, Nuevo León; Tuxpan and

Ciudad Guzmán, Jalisco, and Irapuato, Guanajuato, in Mexico. The samples were labeled, transported to Parasitology Department at UAAAN. The samples were disinfected during 20s with ethanol at 70% of concentration, and then for 10 min in a sodium hypochlorite solution at 2%. Finally the samples were severely washed with sterile aqueous sodium chloride (NaCl) solution. The samples (roots, leaves and stems) which supposedly present bacterias were dissected aseptically into small segments and macerated in a NaCl solution at 0.85%. Tissue extracts were serial dilutes (1:10, 1:100, 1:1000 and 1:10000) and planted on King´s B-agar (Sigma-Aldrich, TOLUCA, MÉXICO) media in triplicate, to recover bacteria present in the plant tissues. Bacteria were grown on Petri dishes at 28°C for 24 – 72 h (Trotel-Aziz et al., 2008). Colonies isolated were store in a sterile glycerol solution at20%at -20°C.

2.4. DNA Extraction

Bacterial strains were cultured for three days on nutrient dextrose agar (NDA). Genomic DNA was extracted as previously described by Frederick et al., 1988 and DNA concentration measured with a Genova Nano Life Science Spectrophotometer, USA.

2.5. Polymerase chain reactions (PCR) amplifications

Primers and probe were specific based on the conserved sequence of theInternal transcribed spacer(ITS) gene by *Clavibacter michiganensis* subsp. *michiganensis* were use at 55°Calignment temperature, Cmm5 forward 5'- GCGAATAAGCCCATATCAA-3' and Cmm6 reverse 5'-CGTCAGGAGGTTCGCTAATA-3' primers that yield an amplicon size of 614 (Caliset al., 2012), for *R. solanacearum* used PEHA3 forward 5'-CAGCAGAACCCG CGCCTGATCCAG- 3' and PEHA6 reverse 5'-ATCGGACTTGATGCGCAGGCCGTT-3' at 70°C by alignment temperature with a 504 bp (Wang et al., 2010), for *X. axonopodis* the primers were BSX1 forward 5'-TCGCCCATAGTCACCATTGGATAGACC-3' and reverse BSX2 5'-GCCTACGCCGAGAAGTTAGCCACCGAG-3' (Cuppelset al*.,* 2006) at 63°C

alignment temperature by 425 bp for expected length; in other hand *P. syringae* employed primer B1 forward 5´-CTTTCCGTGGTCTTGATGAGG-3´ and reverse primer B2 5´-TCGATTTTGCCGTGATGAGTC-3´ primers with at 60°Cwith an expected product of 752 bp (Sorensen et al., 1998) and finally the oligonucleotide sequences by *A*. *tumefacensis* were used VirD2A forward 5'-TCGTCTGGCTGACTTTCGTCATAA-3' and reverse virD2E 5'-CCTGACCCAAACATCTCGGCTGCCCA-3' at 50 °C with a 338 bp by the last product (Tolba and Soliman, 2014).

All reactions were carried out in a final volume of 25 µl containing: 2.5 µl 10×PCR buffer, 3 mM MgCl2, 0.5 mMdATP, dCTP, dTTP, dGTP, 0.4 µM forward and reverse primers, 0.8 µM probe, 1 U Taq polymerase and 1 µl DNA template. Sterile molecular biology grade water. Reactions were run for 40 cycles as follows: 95°C for 10 min, 94°C for 15 sec, and respective temperature alignment for 1 min (Zhao et al., 2007). The amplified products were separated on 1.5% agarose gels including ethidium bromide (0.5 µg mL^{-1}) for 2 h at 6 V cm^{-1} constant voltages in Tris/Borate/EDTA (TBE) buffer (Caliset al., 2012).

2.5.2 Preparation of bacterial suspension

For *in vitro* assays, bacterial suspensions of *C. michiganensis*, *R. solanacearum*, *X. axonopodis, P. syringae* and *A*. *tumefacensis* were prepared in nutrient broth medium on Appleton Woods Orbit 300, Labnetshaker (Kings Norton, Birmingham) incubator at 26°C ± 1°C for 24 h (Fatmi and Shaad, 2002). Suspensions were adjusted to concentration of approximately $1x10^8$ colony forming units (CFU) mL^{-1}, according to McFarland standards, which corresponds to a wavelength of 600 nm equal to 0.283 (A_{600nm}=0.283)(Fontenelle et al., 2011).

2.5.3 Bacteria inhibition microplate assay

For this assay, round shaped well bottom microplates (96-wells) (Ultra Cruz Plate, USA) were used. A sample of 100 µL of nutrient broth (BD bioxon), supplemented with2,3,5-triphenyltetrazolium chloride (TTC, tetrazolium red, Sigma T-8877, St. Louis, USA.) indicator at 0.01% (w/v),were added to each well except on wells of column one. The powder plant extracts samples were resuspended in water or ethanol respectively at an initial concentration of 2000 mg/L, to add 100 µl in wells of columns four of the microplate to carry out the serial dilution were serially diluted up to 50% with water in 96 well microtiter plates (1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9mg/L) by aqueous extract samples were added 100 µl in well of column four of the microplate to carry out the same serial dilution. Bacterial suspension, previously prepared, were seeded in the wells of microplate at 100µlexcept for the wells of the first column, bacteria concentration contained about $1x10^8$colony forming units (CFU) CFU/ml. The third column of the microplates columns contains the medium, TTC, as well as water or ethanol depending on the extract to be tested, as well as bacteria without any sample of plant extracts, in order that this well behave as the control of the evaluation. Immediately, the microplates were covered with its lid and incubated at 28°C overnight. The absorbance of bacteria inhibition microplate assay was read at 540 nm in a microplate reader (Thermo Scientific Multiskan GO, USA) controlled with Thermo Scientific SkanIt software. The assay test was carried out in triplicates for each bacteria. The percent of bacteria inhibition (%) was calculated by the following equation:

$$\% \text{ Inhibiton} = ((A_{control} - A_{sample}) / A_{control})*100$$

Where $A_{control}$ is the absorbance of the column three of all lines and A_{sample} is the rest absorbance of the samples from column four to twelve. The IC_{50} which is the concentration of the samples extracts tested to require inhibit 50% of the bacteria was determined for each sample.

2.6. Fourier Transformed Infrared analysis (FTIR)

The *L. graveolens* and *O. vulgare* extracts that showed the highest antibacterial results were selected for its analyses by FTIR. The *Lippia graveolens* and *Origanum vulgare* extracts did not require any special treatment for the analysis, the sample was run directly in a FTIR spectrophotometer(Nicolet iS 10, USA), using Smart iTR ATR sampling accessory with diamond tip model GX00 at room temperature at range 600 – 4000 cm^{-1} and 100 scans by spectrum in triplicates (Galtieret al., 2007).

2.7. Statistical analysis

The experiment TPC, TFC, DPPH, % inhibition bacteria assays were established under completely randomized design with three repetitions and sixteen treatments. Analysis of variance (ANOVA) and mean test were performed by Tukey test (p=0.05) using the SAS-PC System for Windows. A Probit analysis was performed to determine the 50% inhibitory concentration (IC_{50}) of each extract. Data were analyzed using SAS V 9.0. software for windows. The IC_{50} values were statistically calculated and associated with Chi-square values significant at p<0.05 level.

3. Results and discussion

3.1Mineral content

The mineral compositions of extracts, the results of the analyses were established to give nutrient values per 100 grams of used portion of dried weight. It was observed that the mineral elements varied widely depending of the solvent and tissues of each plant (Table 1).Results for K and Ca were high in sample extracts, in some cases Na and Fe are high, but the most part these elements remain similar in the extracts. Sanchez-Castillo (1988) showed values of 994 mg/100g for K and 1077 mg/100g for Ca from *L*. *graveolens* of Mexican origin, these values were lower than those reported in the present study; also, this author reported for Fe and Na similar values to the obtained in this research. On the other hand, mineral composition of *O. vulgare* reported were higher than those

obtained by Sanchez-Castillo(1998);however, Özcan (2004) reported values higher than those obtained in the present study for Ca (1047.3 mg/100g) and K (1926.5 mg/100g).

It can be noted, in general, that the stems of *L. graveolens* and *O. vulgare* showed higher mineral content than the leaves. Rahmatollah and Mahboeh; (2010) in *Metha* spp.; reported concentration where Ca and Fe were reported in a lower concentration in stem than leaves, K was reported higher concentration sin stem than leaves; the trace elements found in living organism may be essential, that this, indispensable for growth and health, or they maybe nonessential, fortuitous reminders of our geochemical origins or indicator of environmental exposure. The elevated presence of some trace elements in the samples extract can be antinutritional or toxicity by other organism, that was the importance of evaluating the mineral composition in this study.

3.2 Phytochemical analysis

3.2.1 Phytochemical screening

The actual study of the phytochemical analysis components of an extract are important due to the different extractive components used to obtain the desired properties in the plants, as well as the diversity of variables used to carry out the extraction of these components, however this makes it difficult to compare research results for the variety analyzed, nevertheless the techniques handle results in common as those found in Table 2 were showed the phytochemical analysis of *L. graveolens* and *O. vulgare;* It can be observed that in most of the *L. graveolens* and *O. vulgar*e extracts the presence of flavonoids, quinones, saponins, sterols, terpenes and tannins (Table 3). The presence of these compounds have been also reported for *O. vulgare* extracts, obtained with the same solvents (Babuet al., 2007) the same presence of some biological active compounds like glycosides, carbohydrates, oils, phenolic compounds, protein, aminoacids, saponins, quinones, tannin and terpenpoids of different solvent extract like aqua, ethanol, methanol, benzene and chloroform in *Origanum vulgare* against phytopathogenic bacteria *Xanthomonas.* Another

phytochemical screening with antimicrobial activity of , Dahiya and Purkayastha(2012), reported for *Lippia* spp. extracts with ethanol and water the presence of compounds, such as reducing sugars, tannins, glycosides, sterols and saponins, similar to those found in this research(Dahiya and Purkayastha, 2012).

3.2.2. Total phenolic content

In general the TPC, for both tissues of *O. vulgare* and *L. graveolens*, ranged from 8.97 to 123.93 GA mEq/g of dry weight (Table 3). The extracts from *L. graveolens* showed higher TPC than *O. vulgare*. It have been reported, values of 102.6 mg/g and 42.7 mg/g for L. graveolens ethanol and water extracts, respectively; and for O. vulgare, values of 33.2 and 20.6 mg/g with ethanol and water extracts, respectively (Karimi et al.,2015). It observed that found lower levels in ethanloic extracts of Mexican *Lippia graveolens* with 102.6 mg/g on ethanloic extracts and 33.2mg/g of European Oregano, by water extracts the results obtained were lower than those found in this research. The water and ethanol extracts of *Origanum vulgare* were studied as high antimicrobial against *Helicobacter pylori* were total phenolic results on 1.04 mg/g and 3.24 mg/g respectively that show an effective results against bacteria control (Chun et al., 2005). Lin et al. (2004) reported phenol content with value 19.1 mg/g against inhibitions of *Listeria monocytogenes* of oregano extract, which results are similar at phenol totals of *Origanum vulgare* by this study. Therefore, *L. graveolens* and *O. vulgare* could be used as a natural source of phenolic compounds.

3.2.3. Total flavonoid content

The TFC results showed the highest value for L.*graveolens* ethanol extract of stems, with 161.62 mg of QE/ g dry weight plant material; followed by the leaves water extract of the same species with 160.49 mg/g.; another way the lowest level was 7.66 mg/g Lg.S.H.C. that are too similar at Ov.S.H.C.9.13 mg/g. The results obtained were higher that the reported by Babili et al.(2011), who obtained a value of 0.014 mg/g mg of QE/ g dry weight plant material from oregano ethanol

extract.by an ethanolic extract of Oregano that showed antimalarial and antioxidant activity (Babili et al., 2011), other study by Rocha et al. (2008) reported similar values for *L. graveolens* extracts to those reported in this study, in Table 3the origin of these extracts were for *L.graveolens* of Puebla and Queretaro city on Mexico that shows antioxidant and antimutagenic activity (Rocha et al. 2008). Another study For O. vulgare ethanol extract, Licina et al. (2013) reported a TFC value higher than the obtained in this study (135 mg/g). of *Origanum vulgare* by southwest Serbia as well as to evaluated antioxidant activity and antimicrobial activity of human pathogenic and food spoilage bacterial and fungi (Licina et al., 2013), the result were similar at this study by 122.85g /mg of *Origanum vulgare* stem EtOH powder.

3.2.4. Antioxidant Activity

The powder extracts of aqueous by *L. graveolens* extracts and powder of *O. vulgare* leaves ethanol extracts showed high antioxidant activity (Table 3)96.57% on leafs, 94.31 %on stem and 94.46%, respectively) in the DPPH assay. Table 3 contain the lower ranged obtained on antioxidant activity assay, which corresponds at aqueous extract of *Origanum vulgare* 0.17% and 0.18% by leaf and stem. These values obtained were Chun et al. (2005) reported values of 80% and 82% of DPPH inhibition from ethanol and water extracts of commercial oregano, also Gonzalez et al. (2007) reported an antioxidant activity of 87.6%for *L. graveolens* water extract. These results are lower than the reported in this research for *L. graveolens* and O*. vulgare*. Lin et al. (2009) reported 86.66% of DPPH inhibition from ethanol extracts of *O.vulgare*, similar reported by Chun et al. (2005) and Gonzalez et al. (2007). The high antioxidant capacity showed by *L. graveolens* and *O. vulgare* demonstrates the potential of these plants as a natural antioxidant agent as antibacterial capacity, furthermore considering that other species of the same genus and chemical composition has been used successfully as antifungal and antiviral agents.

3.3 Polymerase chain reaction (PCR)

PCR detection in bacteria's suspensions confirmed the species being sought. By *A. tumefacensis* the primersVirD2A/VirD2E produced the expected DNA products of 338 base pairs (bp), for *C. michiganensis* the expected DNA product of 614 bp for Cmm5/Cmm6 primers. On other hand *P. syringae* presented a product of 752 bp for primers B1/B2; *R. solanacearum*, displayed a figment of 504 bp for PEHA3/PEHA6 primers and finally *X. axonopodis,* showed a product of 425 bp for primers BSX1/BSX2.

3.4 Effect of L. graveolens and *O. vulgare* against *phytopathogen bacteria.*

The antibacterial effect of *L. graveolens* and *O. vulgare* extracts is expressed as Inhibition Concentration 50(IC_{50}) in µg/mL (Table 4).It has been reported that lower values of $IC_{50}$are indicative of a higher antibacterial activity. The antibacterial effects of the best treatment against *A. tumefacensis* was Lp.L.E.C. with an IC_{50}of19.01µg/mL.. Ahmad et al. (2016) evaluated various fractions of *Rumexhastatus* D.Don. as a potential antitumor and anti-angiogenic against *A.tumefaciens* reporting high levels by$IC_{50}$of 1138.9 µg/mL on aqueous extract. For *C.michiganensis* the best inhibition was achieved with an IC_{50} of12.71µg/mL from Lp.S.E.P. Romero et al. (2015) reported $IC_{50}$of 833.33 54.17 and 23.14 µg/mL as IC_{50} of methanolic extracts of *A.multifidi* and Penicillin and Streptomicin respectively; the last two drugs were used as controls. Otherwise, Ov.S.E.P, had the highest inhibition against *P.syringe* with an IC_{50}value of14.03µg/mL; this concentration contrast with those used by Son et al. 2007 that by using Bikaverin and fusaric acid required a >100 and 11 µg/mL respectively, where Bikaverin requires high levels for control *P.syringae*, but Fusaric acid required less concentration than Ov.S.E.P, however the cost of both products in the market are high, which represents a limitation for the effective control of *P.syringae.* The highest inhibition of *R. solanacearum* was achieved by Ov.S.H.C extract with an IC_{50} of 64.49 µg/mL. In addition, an IC_{50}value of 2.03 µg/mL was required from Lg.S.E. extract to inhibit *X.*

*axonopodis, t*he inhibition of *R. solanacearum* and *X. axonopodis* has been also reported by Al-Sanfi (2017), with *Euphorbia hirta* methanol extract with IC_{50}values of 40 µg/mL and 640 µg/mL, respectively. These values were higher than those obtained with *L. graveolens* in this study.

The antibacterial activity that *L. graveolens* and *O. vulgare* extracts exhibited could be attributed to the TPC, TFC and antioxidant capacity identified in the extracts (Masmoudi-Allounce et al., 2016), and also, to different families of compounds revealed on the phytochemical screening, mainly: tannins, flavonoids, sterols and terpenes, quinones and saponins (Obouayeba et al., 2014; Medoatinsa et al., 2016; Mujeeb et al., 2014; Koudoro et al., 2014). However, it is important to carry out studies on the identification of the bioactive compounds of L. graveolens and O. vulgare that could promotes the antibacterial effect of the extracts. By the above, the process reported to obtain the extracts, is an alternative to recover bactericides and antioxidants natural agents, an added value compared with other processes that requires extra steps by purification that increase the cost of the development technologies.

3.5 FTIR

The observed bactericide activity of *Lippia graveolens* and *Origanum vulgare,* could be attributed to the presence of the phytochemical compounds analyzed on this research. The FTIR Spectroscopy is not capable to determine exactly the main compound of the present extract, but manifest what it's the more abundant chemical compound. The best results of the IC_{50} were analyzed by infrared spectroscopy, the selected extracts for the FTIR analysis were: Lg.L.E.C. extract (Figure 1), Lg.S.E.P. (Figure 2), Ov.S.E.P. (Figure 3) and Ov.S.H.C. (Figure 4). The infrared spectra showed some specific peaks corresponding to groups of alcohols, phenols, aromatics, carboxylic acids, methylenes and benzenes, confirming the presence of chemical compounds. Al-Sheibany et al. (2005) revealed a clear broad band in the region 3382cm-1 due to stretching vibration of phenolic O-H group, at 3020

cm^{-1} due to aromatic C-H stretching, 2960-2868 cm^{-1} due to C-H stretching branched alkane, 1585-1458 cm^{-1} due to C-C ring stretching band, 1458-1421 cm^{-1} due to the OH bending vibration, show a peak at 1359 cm^{-1} due to the isopropyl group, a strong band in 1251 cm^{-1} due to C-O stretching, 800 cm^{-1} due to aromatic C-H bending, *L. graveolens* leaves ethanol crude extract, *L. graveolens* stems ethanol powder extract and *O. vulgare* stems ethanol powder extract, are in agree with the description mentioned above. On the other hand, *O. vulgare* stems water crude extract showed similar groups to the reported by Pensel et al. (2014) for thymol, with signals shown exhibits a broad band appearing at 3250-3300 cm^{-1} assigned to the stretching vibration of OH group, for stretching ofCH_3 group at 2962-2867 cm^{-1}due to stretching of CH_3 group, and for C-C ring stretching a signal at1458-1419 cm^{-1}due to the C-C ring stretching were observed. In the region 1380-1340 cm^{-1}due to O-H in-plane bending vibration, 1286 cm^{-1}due to isopropyl group region, astrong band 1244 cm^{-1} due to C-O stretching in phenol produce region, 800cm^{-1} out-of-plane due to the aromatic C-H bending. Further investigations are necessary to confirm specific compounds.

4. Conclusions

L. graveolens and *O.vulgare* extracts exhibited antibacterial activity against *C. michiganensis*, *R. solanacearum*, *and X. axonopodis, P. syringae* and *A*. *tumefacensis*. Leaves and stems extracts of *L. graveolens* and *O. vulgare* presented high phenolic and flavonoid contents, and antioxidant activity. Further investigations are necessary to identify the chemical compounds that promotes the antibacterial activity, confirm specific compounds and to improve the extraction and analyze the application form in the crop, also to determine toxicity and side effects of active constituents of the extracts, providing support to improve and development of new products with more bioactive efficiency and friendly by environmental. *L. graveolens* and *O. vulgare extracts* represent an alternative to be used as botanical bactericides, which can substitute the use of the synthetic products.

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Table 1. Chemical composition of Fe, K, Na and Ca, of *Lippia graveolens* and *Origanum vulgare* extracts

Plant extract	Fe	K	Na	Ca
	mg/100 g per plant of used portion of dried weight			
Lg.L.H.P.	3.0	3009	30	2227
Lg.S.H.P.	1504	12938	318	1486
Lg.L.E.P.	0.7	636	29	29
Lg.S.E.P.	10	749	54	15
Lg.L.H.C.	3.2	3998	30	2235
Lg.S.H.C.	1364	12942	319	1492
Lg.L.E.C.	0.9	636	31	30
Lg.S.E.C.	10	750	54	15
Ov.L.H.P.	2.7	12412	113	7375
Ov.S.H.P.	12	25471	255	4362
Ov.L.E.P.	0.8	3829	121	40
Ov.S.E.P.	73	17995	8375	2527
Ov.L.H.C.	2.8	12462	115	7376
Ov.S.H.C.	13	25480	257	4368
Ov.L.E.C.	0.8	3826	123	986
Ov.S.E.C.	73	18005	8395	2529

Table 2. Preliminary phytochemical screening analysis of *Lippia graveolens* and *Origanum vulgare*

Plant extract	Alkaloids		Carbohydrates	Carotenoids	Coumarines	Flavonoids	
	D	S				S (flavanol)	NaOH
Lg.L.H.P.	-	-	-	+	+	Flavanol	Flavanone
Lg.S.H.P.	-	-	+	-	+	Flavanol	Flavanone
Lg.L.E.P.	-	-	+	-	+	Flavanol	Flavanone
Lg.S.E.P.	-	-	-	+	-	Flavanol	Flavanol
Lg.L.H.C.	-	-	-	+	+	Flavanol	Flavanol
Lg.S.H.C.	-	-	-	-	+	Flavanol	Flavanol
Lg.L.E.C.	-	-	-	-	+	Flavanol	Flavanone
Lg.S.E.C.	-	-	-	+	-	Flavanol	Flavanone
Ov.L.H.P.	-	-	-	-	+	Flavanol	Flavanone
Ov.S.H.P.	+	+	+	-	-	Flavanol	Xanthone
Ov.L.E.P.	-	-	-	-	+	Flavanol	Flavanone
Ov.S.E.P.	+	+	+	-	-	Flavanol	Xanthone
Ov.L.H.C.	-	-	-	-	+	Flavanol	Flavanone
Ov.S.H.C.	+	+	+	-	-	Flavanol	Xanthone
Ov.L.E.C.	-	-	-	-	-	Flavanol	Flavanone
Ov.S.E.C.	+	+	+	-	+	Flavanol	Xanthone

Extract plant	Free reducing sugars		Glycosides	Polysaccharides	Purines	Quinones		
	F	B				NH4OH	H2SO4	B
Lg.L.H.P.	-	-	-	-	-	-	Antraquinone	Benzoquinone
Lg.S.H.P.	-	-	-	-	-	Antraquinone	Antraquinone	Benzoquinone
Lg.L.E.P.	+	+	-	-	+	Antraquinone	Antraquinone	Benzoquinone
Lg.S.E.P.	+	+	-	-	+	Antraquinone	Antraquinone	Benzoquinone
Lg.L.H.C.	-	-	-	-	-	Antraquinone	Antraquinone	Benzoquinone
Lg.S.H.C.	-	-	-	-	-	Antraquinone	Antraquinone	Benzoquinone
Lg.L.E.C.	+	+	-	-	-	Antraquinone	Antraquinone	Benzoquinone

Lg.S.E.C.	+	+	-	-	-	Antraquinone	Antraquinone	Benzoquinone
Ov.L.H.P.	-	-	-	-	+	Antraquinone	Antraquinone	Antraquinone
Ov.S.H.P.	-	-	-	-	+	-	-	-
Ov.L.E.P.	+	+	-	-	+	Antraquinone	Antraquinone	Antraquinone
Ov.S.E.P.	-	-	-	-	-	-	-	-
Ov.L.H.C.	-	-	-	-	-	Antraquinone	Antraquinone	Antraquinone
Ov.S.H.C.	-	-	-	-	-	-	-	-
Ov.L.E.C.	+	-	-	-	-	Antraquinone	Antraquinone	Antraquinone
Ov.S.E.C.	-	-	-	-	-	-	-	-

Extract plant	**Saponins**	**Sterols and Terpens**		**Soluble starch**		**Tannins**	
	Foam	**Liberman-Burchard**	**Rhosenthaler**	**KOH and H2SO4**	**Gelatin**	**FeCl3**	**K3(Fe(CN)6)**
Lg.L.H.P.	+	Sterol	-	+	+	Catechol	Phenols
Lg.S.H.P.	+	Terpenoid	Triterpenoids	+	+	Catechol	Phenols
Lg.L.E.P.	+	Sterol	-	-	+	Catechol	Phenols
Lg.S.E.P.	+	Sterol	-	-	+	Catechol	Phenols
Lg.L.H.C.	+	Sterol	-	+	+	Catechol	Phenols
Lg.S.H.C.	+	Terpenoid	Triterpenoids	-	+	Catechol	Phenols
Lg.L.E.C.	+	Sterol	-	+	+	Catechol	Phenols
Lg.S.E.C.	+	Sterol	-	+	+	Catechol	Phenols
Ov.L.H.P.	+	Terpenoid	Triterpenoids	-	+	Catechol	Phenols
Ov.S.H.P.	+	Sterol	-	-	+	Gallic acid	Phenols
Ov.L.E.P.	+	Terpenoid	Triterpenoids	+	+	Catechol	Phenols
Ov.S.E.P.	+	Sterol	-	+	+	Gallic acid	Phenols
Ov.L.H.C.	+	Terpenoid	Triterpenoids	+	+	Catechol	Phenols
Ov.S.H.C.	+	Sterol	-	-	+	Gallic acid	Phenols
Ov.L.E.C.	+	Terpenoid	Triterpenoids	+	+	Catechol	Phenols
Ov.S.E.C.	+	Sterol	-	+	+	Gallic acid	Phenols

(+) indicates presence; (-) indicates absence

Table 3. Total phenols and flavonoids extraction and Antioxidant capacities of the analyzed *Lippia graveolens* and *Origanum vulgare* samples

Plant extract	TPC (GA mEq/g dry weigth)	TFC (Q mEq/g dry weigth)	Antioxidant activity DPPH (%)
Lg.L.H.P.	110.85 ± 0.05 ab	160.49 ± 0.04 ab	96.57± 0.39 b
Lg.S.H.P.	53.35 ± 0.84 cdef	74.23± 0.78 ed	94.31± 0.24 a
Lg.L.E.P.	123.93 ± 0.06 a	180.10 ± 0.07 a	42.09± 0.06defgh
Lg.S.E.P.	111.61 ± 0.08ab	161.62± 0.09 ab	55.37 ± 0.01 de
Lg.L.H.C.	40.54 ± 0.04defg	55.70 ± 0.01def	53.29± 0.01def
Lg.S.H.C.	8.97 ± 0.01 g	7.66± 0.01 g	18.77± 0.00efghi
Lg.L.E.C.	64.19 ± 0.23cde	90.50 ± 0.35 dc	13.64± 0.00fghi
Lg.S.E.C.	85.15 ± 0.06bc	146.33 ± 0.04 ab	5.76± 0.01ghi
Ov.L.H.P.	67.79 ± 0.06 cd	95.75 ± 0.05 cd	0.17 ± 0.01i
Ov.S.H.P.	26.37 ± 0.04fg	35.34 ± 0.04efg	0.18 ± 0.01i
Ov.L.E.P.	22.95 ± 0.03fg	28.63 ± 0.03fg	94.46 ± 0.02 c
Ov.S.E.P.	85.76± 0.05bc	122.85 ± 0.04bc	45.67± 0.05defg
Ov.L.H.C.	20.86 ± 0.23fg	25.50 ± 0.12fg	67.53± 0.17 d
Ov.S.H.C.	9.28 ± 0.08 g	8.13 ± 0.01 g	30.84± 0.00defghi
Ov.L.E.C.	29.16 ± 0.02efg	38.50 ± 0.05efg	3.88± 0.01 hi
Ov.S.E.C.	15.52 ± 0.09 eg	17.49 ± 0.15fg	9.37± 0.01 ghi

Data are means of three determinations. Values with the same latter within each column are equal (Tukey, α=0.05%)

Table 4. Plant extracts concentrations IC_{50} (µg/mL) to inhibit *A. tumefacensis* C*. michiganensis, P. syringae, R. solanacearum, and X. axonopodis,* by microdilution assay

Plant extract	*A. tumefacensis* IC_{50}(µg/mL)	*C. michiganensis* IC_{50}(µg/mL)	*P. syringae* IC_{50}(µg/mL)	*R. solanacearum* IC_{50}(µg/mL)	*X. axonopodis* IC_{50}(µg/mL)
Lg.L.H.P.	126.34±0.20 h	509.70 ± 0.15 b	419.50 ± 0.81 e	630.77 ± 0.30 d	395.30 ± 0.83 i
Lg.S.H.P.	116.76±0.17 j	219.30 ± 0.25 l	433.45 ± 0.78 d	341.91 ± 0.28 i	317.31 ± 0.92 m
Lg.L.E.P.	240±0.08 l	74.94 ± 0.44 n	308.01 ± 0.40 i	615.35 ± 0.14 e	449.55 ± 0.45 f
Lg.S.E.P.	147.41±0.08 e	12.71 ± 0.24 o	333.39 ± 0.41 h	437.45 ± 0.14 h	2.03 ± 0.50 p
Lg.L.H.C.	268.69±0.17 c	459.16 ± 0.62 d	484.19 ± 0.45 b	795.43 ± 0.15 b	817.40 ± 0.10 a
Lg.S.H.C.	289.29±0.13 a	294.87 ± 0.14 g	594.29 ± 0.11 a	885.90 ± 0.12 a	532.17 ± 0.12 c
Lg.L.E.C.	19.01±0.11 n	326.97 ± 0.12 f	250.93 ± 0.12 j	318.31 ± 0.12 j	358.28 ± 0.11 k
Lg.S.E.C.	20.05±0.11 m	287.05 ± 0.31 h	234.01 ± 0.57 k	153.48 ± 1.92 n	482.98 ± 0.14 e
Ov.L.H.P.	141.79±0.18 f	380.22 ± 0.18 e	460.70 ± 0.30 c	552.06 ± 0.26 f	328.91 ± 0.88 l
Ov.S.H.P.	124.05±0.33 i	1053.05 ± 0.22 a	406.56 ± 0.69 f	445.79 ± 0.25 g	391.11 ± 0.11 j
Ov.L.E.P.	136.64±0.17 g	281.03 ± 0.83 j	130.82 ± 0.32 n	224.75 ± 0.22 l	95.44 ± 0.55 n
Ov.S.E.P.	96.74±0.08 k	154.58 ± 0.74 m	14.30 ± 0.49 o	307.57 ± 0.14 k	11.73 ± 0.45 o
Ov.L.H.C.	266.06±0.20 d	478.62 ±0.15 c	394.69 ± 0.20 g	683.97 ± 0.19 c	684.64 ± 0.13 b
Ov.S.H.C.	270.1±0.16 b	277.55 ± 0.14 k	593.78 ± 0.64 a	64.49 ± 0.16 o	490.74 ± 0.74 d
Ov.L.E.C.	20.34±0.12 m	287.42 ± 0.22 h	218.26 ± 0.06 m	181.73 ± 0.08 m	419.89 ± 0.10 h
Ov.S.E.C.	23.54±0.15 l	283.00 ± 0.033	229.17 ± 0.07 l	154.82 ± 1.93 n	432.52 ± 0.13 g

Data are means of three determinations. Values with the same latter within each column are equal (Tukey, α=0.05%)

Figure 1. Infrared analysis of *Lippia graveolens* leaf EtOH crude extract

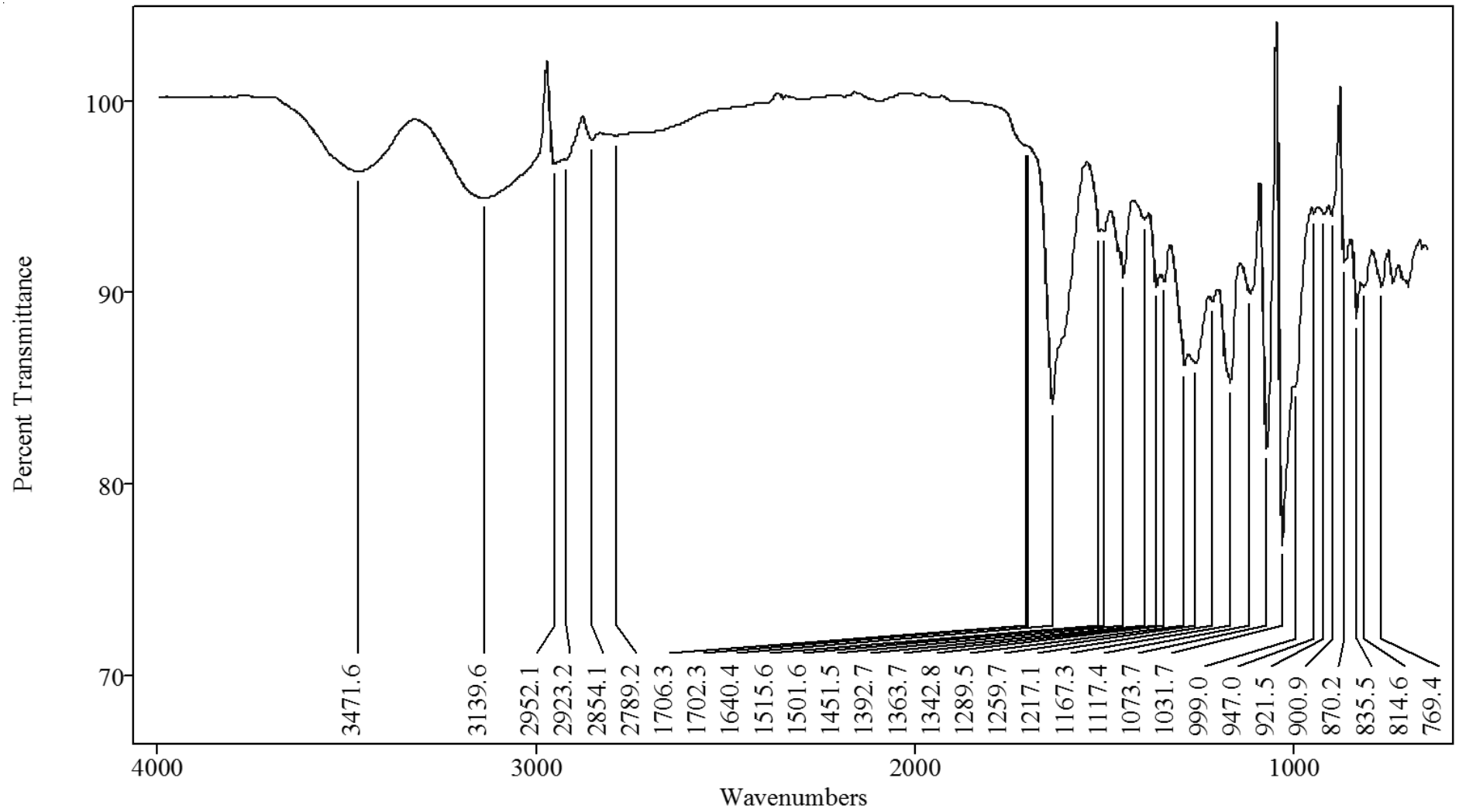


Figure 2. Infrared analysis of *Lippia graveolens* stem EtOH powder

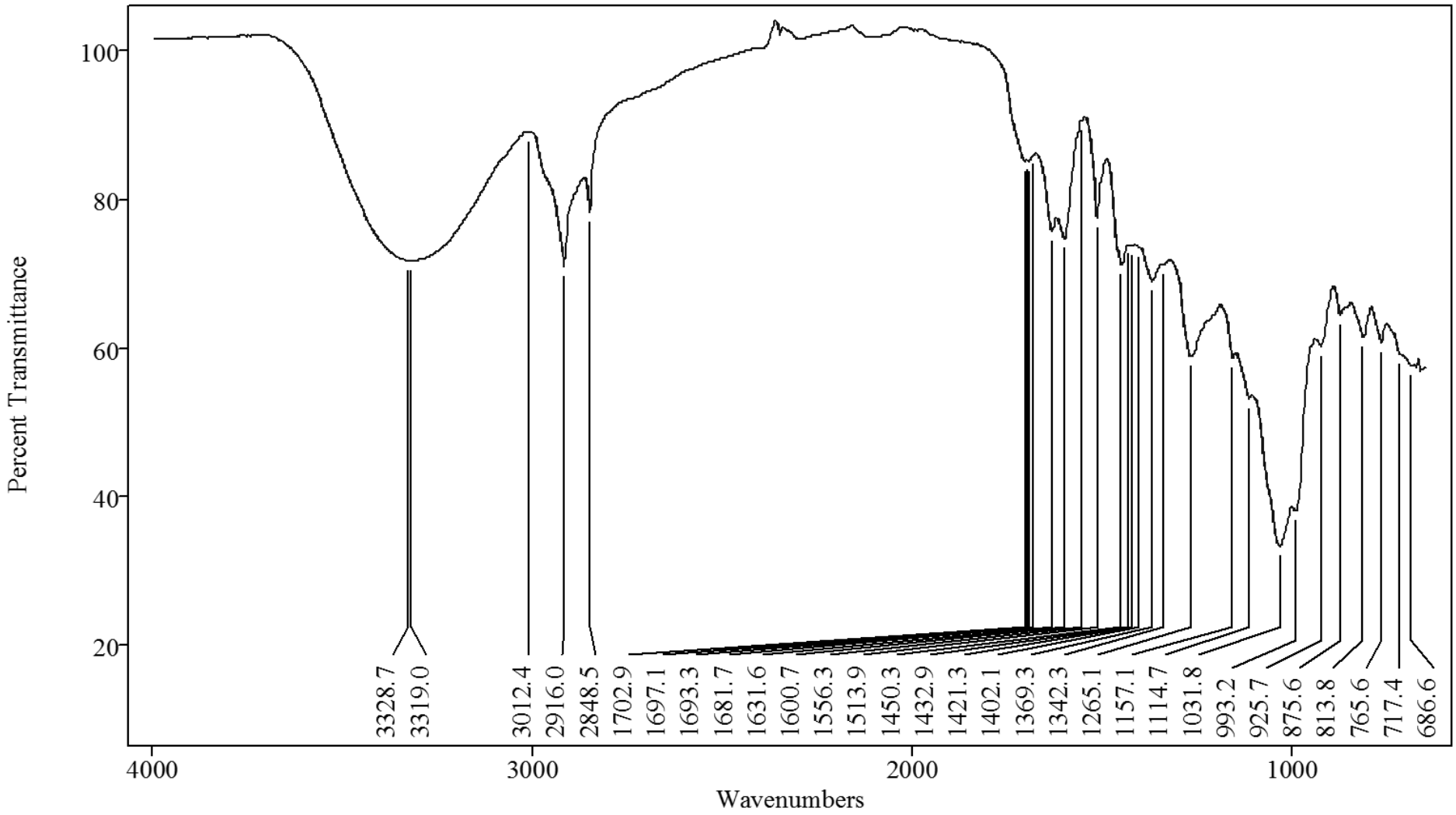


Figure 3. Infrared analysis of *Origanum vulgare* stem EtOH powder

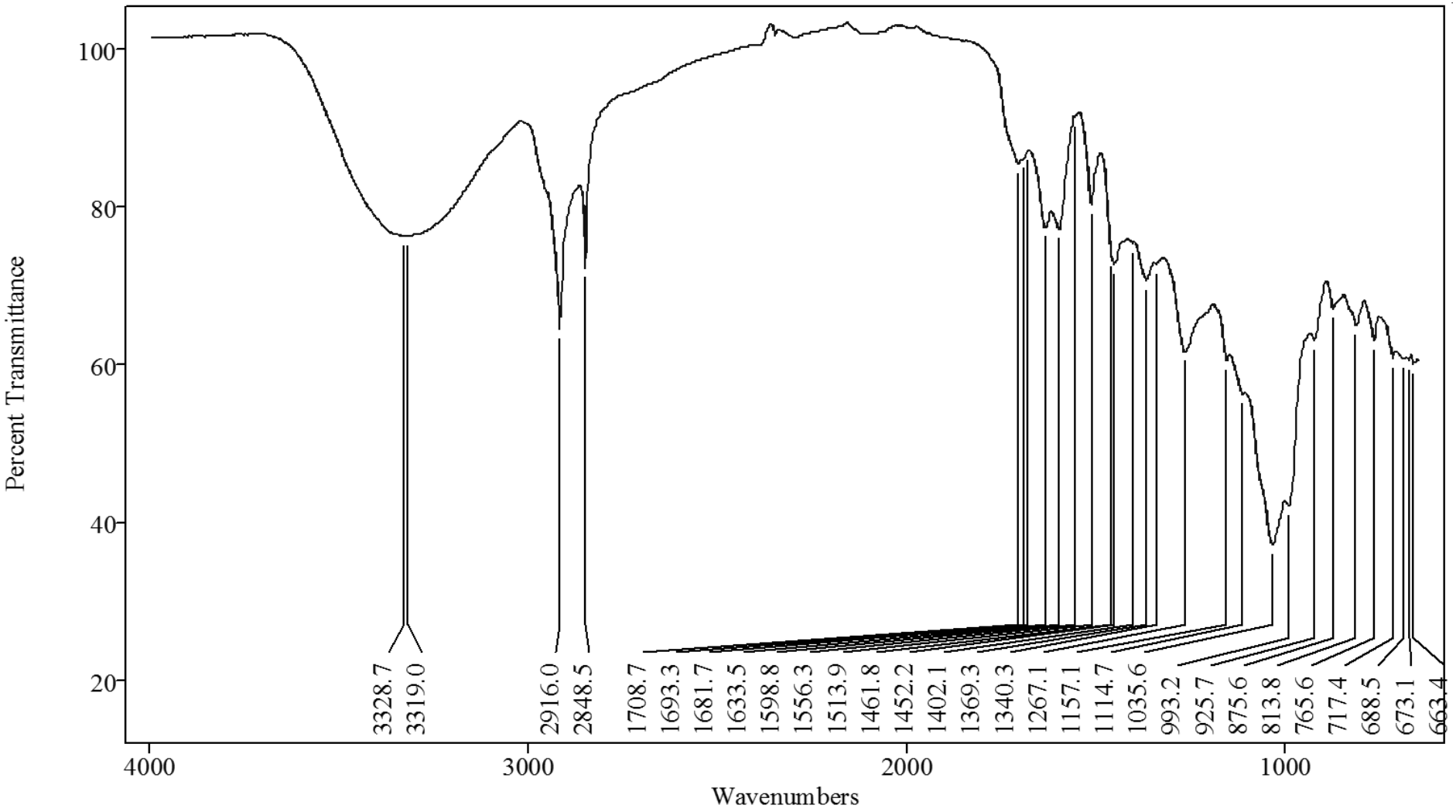
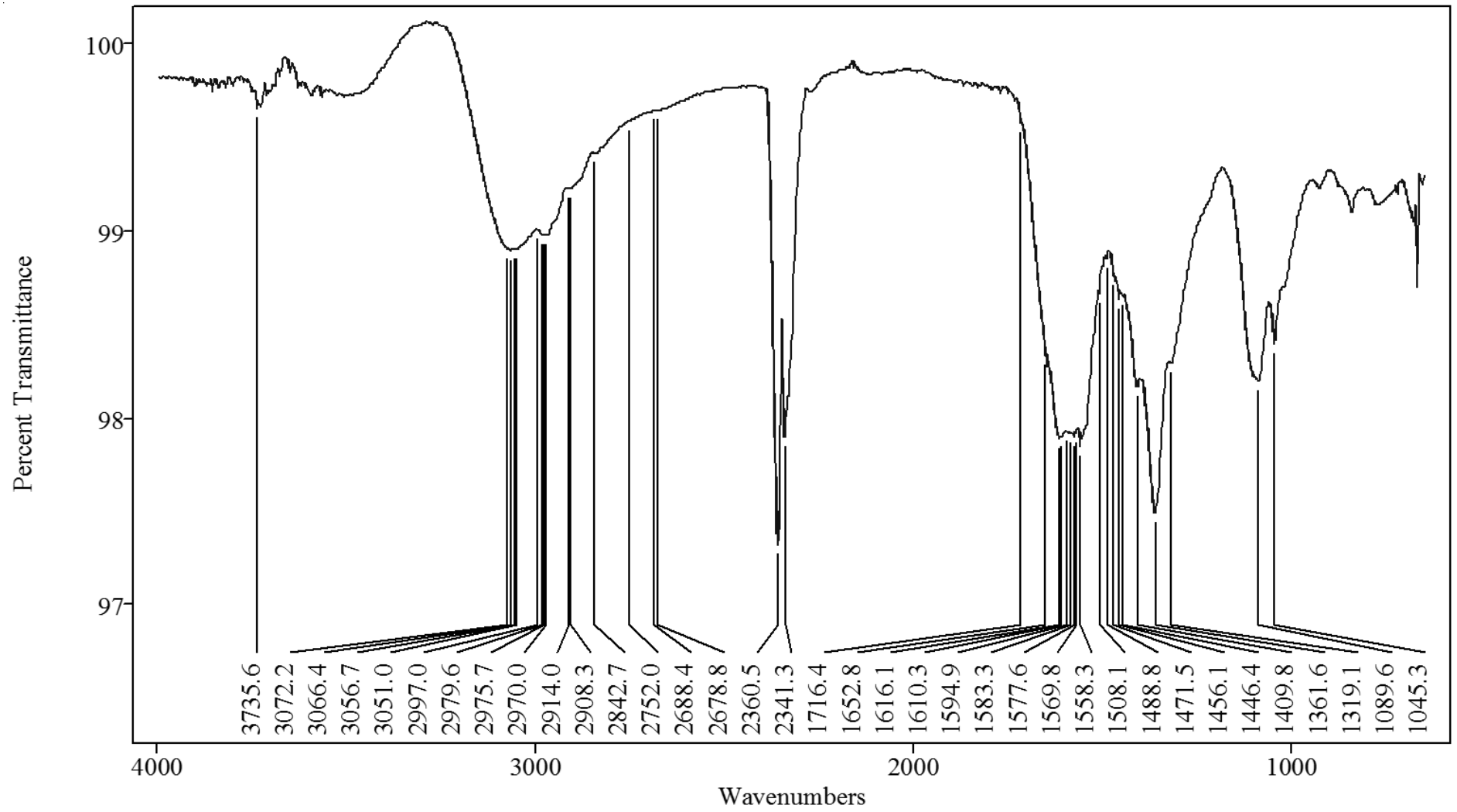


Figure 4. Infrared analysis of *Origanum vulgare* stem H_2O crude



Artículo III

Effect of Mexican desert plants with metabolites of *Bacillus subtillis* against phytopathogen bacteria's of Tomato in Green house

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Abstract

Tomato is one of the most popular and important vegetable crops with commercial value in the world. Bacterial pathogens are serious problem no tomato crop. Bacterial canker, wilt and spot caused respectively by the phytopathogens *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum* and *Xanthomonas axonopodis*, affect tomato production. The present study, showed the effects of three products for the bactericida control on tomato crop in vitro and in vivo on greenhouse against *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum* and *Xanthomonas axonopodis*, the first product consists of a mixture of ethanol extracts from *Lippia graveolens*, *Larrea tridentate, Carya illinoensis* and *Jatropha dioica*, second product has the same extracts as metabolites of *Bacillus subtilis*, and third product denominated Biobacter O® from the company Greencorp as a commercial control. The most significant reductions of severity were conferred by the second product (75%) against *C. michiganensis,* and (50%) against *R. solanacearum*; first and second product reduce the severity with 25% against *X. axonopodis*.

Key words: Tomato, Bacterial pathogen, Mexican desert plants, IC_{50}, biocontrol.

1. Introduction

The tomato (*Solanum lycopersicum)* is one of the most popular and important vegetable crops with commercial value in the world (Janahiraman et al., 2016). In 2014, Mexico sowed 95207 ha and produced 3536305 tonnes of fresh tomato (FAOSTAT, 2014) which placed it at the first global exporter of this vegetable after Netherlands (SAGARPA, 2010). There are several factors that decrease tomato production, including diseases caused by fungi, bacteria, and viruses (Terumi-Itako et al., 2015).

Bacterial canker, wilt and spot caused respectively by the phytopathogens *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum* and *Xanthomonas axonopodis* (Paulin et al., 2017; Gyu-Lee et al., 2017; Herrera-Diaz et al., 2016). Disease control is difficult because of absence of commercially acceptable resistant tomato cultivars (Herrera-Diaz et al., 2016). Studies focusing on bacterial chemical control are unusual and afford variable results. With respect to standard bactericides, secondary spread of the pathogens in the field can only be reduced by treating seedlings with streptomycin, ampicillin, tetracycline, penicillin and copper compounds, however, some bacteria's presence a high tolerance to copper (Bastas, 2014; Mbega et al., 2012; Abo-Elyousr & Asran, 2009). This situation has directed so many researchers to control the diseases in ecologically sound methods based on disturbing the host-pathogen relations ((Bastas, 2014).

The natural plants products has a number of compounds with antibiotic and antimicrobial properties (Abo-Elyousr & Asran, 2009). Many reports revealed that, plant metabolites and plant pesticides appear to be one of the better alternative as they are known to have minimal environmental impact and danger to consumer in contrast to synthetic pesticides (Narasimha et al., 2013). The bactericidal effect have been attributed to the antioxidant compounds which give antimicrobial activity to the plants (Zengin and Baysal, 2014). The use of biofertilizers is an alternative to improve the conditions of Mexican fields and world-wide (Villareal-Sanchez et al., 2003). There are few studies on Mexican oregano (*Lippia graveolens* H. B. K.) that measure the antimicrobial activity of its essential oil (Arana-Sánchez et al., 2010; Martínez-Rocha et al., 2008). On the other hand, several studies has been on screening of antifungal and antibacterial activity plants of Mexico as *Larrea tridentata* (Osorio et al., 2010; Hernández-Castillo et al., 2008)*, Jatropha dioica* (Alanís-Garza et al., 2007; Silvia-Belmares, et al, 2014) and *Carya illinoensis* (Cruz-Vega et al., 2008).

Another way numerous species of soil bacteria which flourish in the rhizosphere of plants or around plant tissues stimulate plant growth and reduce phytopathogen population by antagonistic behavior. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria) (Almaghrabi et al., 2013). *Pseudomonas* spp. and *Bacillus* spp., colonize roots of monocots and dicots, directly or indirectly promote plant growth, and elicit induced systemic resistance (Yong-Soon et al., 2015). Some treatments with *Bacillus cereus* increased shoot height but not shoot biomass, and elicited induced systemic resistance to *Xanthomonas axonopodis* pv. *vesicatoria* in pepper (*Capsicum annuum*) (Yang et al.,2009) and *Pectobacterium carotovorum* subsp. *carotovorum* in tobacco (Yang et al.,2011). Although extensive research indicates that PGPR influence plant growth, development, and disease resistance in a variety of plant species (Yong-Soon et al., 2015).

In the present study, were examined the effects of three products for the bacterial control on tomato crop in green houses against *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum* and *Xanthomonas axonopodis*, the first product consists of a mixture of ethanol extracts from *Lippia graveolens*, *Larrea tridentate, Carya illinoensis* and *Jatropha dioica*, second product has the same extracts as metabolites of *Bacillus subtilis*, and third product denominated Biobacter O® from the company Greencorp as a commercial control.

Material and methods

1.1. Isolation and identification of bacteria

1.1.1.Beneficial Bacteria

The organism tasted in these assay were obtained from the collection of the Laboratory of Phytopathology at Department of Parasitology (Universidad Autónoma Agraria Antonio Narro,

Coahuila, Mexico; UAAAN). The bacteria strain of *Bacillus subtilis* (*B.s* 4) were grown in 50 ml of nutrient broth at 28°C and maintained in nutrient agar slant at 4°C (Latha et al., 2009).

1.1.2.Phytopathogenic Bacteria

C.michiganensis, *R. solanacearum*, and *X. axonopodis* were isolated from the roots, leaves and stems of tomato plants that presented the symptoms of the diseases. The samples were disinfected 20s with ethanol at 70% of concentration, and then for 10 min in sodium hypochlorite solution at 2%. Finally the samples were severely washed with sterile aqueous sodium chloride (NaCl) solution. The samples were dissected aseptically into small segments and macerated in a NaCl solution at 0.85%. Tissue extracts were serial dilutes (1:10, 1:100, 1:1000 and 1:10000) and planted on King´s B-agar media in triplicate. Bacteria were grown on Petri dishes at 28°C for 24 – 72 h (Trotel-Aziz et al., 2008). Bacterial strains were cultured for three days on nutrient dextrose agar (NDA). Genomic DNA was extracted as previously described by Frederick et al., 1988 and DNA concentration measured with a Genova Nano Life Science Spectrophotometer, USA. Primers and probe were specific based on the conserved sequence of the Internal transcribed spacer(ITS) gene by *C. michiganensis subsp. michiganensis* were use at 55° alignment temperature, Cmm5 forward 5'-GCGAATAAGCCCATATCAA-3' and Cmm6 reverse 5'-CGTCAGGAGGTTCGCTAATA-3' primers that yield an amplicon size of 614 (Calis et al., 2012), for *R. solanacearum* used PEHA3 forward 5'-CAGCAGAACCCG CGCCTGATCCAG- 3' and PEHA6 reverse 5'-ATCGGACTTGATGCGCAGGCCGTT-3' at 70°C by alignment temperature with a 504 bp (Wang et al., 2010), for *X. axonopodis* the primers were BSX1 forward 5'-TCGCCCATAGTCACCATTGGATAGACC-3' and reverse BSX2 5'-GCCTACGCCGAGAAGTTAGCCACCGAG-3' (Cuppels et al., 2006) at 63°C alignment temperature by 425 bp for expected length. All reactions were carried out in a final volume of 25 µl containing: 2.5 µl 10×PCR buffer, 3 mM MgCl2, 0.5 mMdATP, dCTP, dTTP, dGTP, 0.4 µM forward and reverse primers, 0.8 µM probe, 1 U Taq polymerase and 1 µl DNA template. Sterile molecular biology grade

water. Reactions were run for 40 cycles as follows: 95°C for 10 min, 94°C for 15 sec, and respective temperature alignment for 1 min (Zhao et al., 2007). The amplified products were separated on 1.5% agarose gels including ethidium bromide (0.5 µg mL-1) for 2 h at 6 V cm-1 constant voltages in Tris/Borate/EDTA (TBE) buffer (Calis et al., 2012).

1.2. Collection of plant material

Leaves of *L. graveolens*, *L. tridentate,* and roots of *J. dioica*, were collected in the municipality of General Cepeda, Coahuila, Mexico (25°22´42.2" N and 101°27´53.1" W); *C. illinoensis* walnut husk were collected in Saltillo Coahuila, México (25°27´35.3" N and 100°54´21.5" W), were collected during summer season (June, 2016). The samples were labeled and transported in brown paper bags inside iceboxes, samples were weighed the same day of the collection, to arrive at the Phytopathology Laboratory from Parasitology Department at the UAAAN. Immediately, leaves and stems were separated. Vegetal tissue were dehydrated at room temperature for 10 days, then was dehydrated using conventional oven Quincy lab, Chicago, USA Inc. at 60°C for 3 days at constant weight, and ground using a miller CUISINART, NJ,USA, using a 1 mm screen. The powder was stored in dark bottles at room temperature until extraction was performed (Castillo et al., 2010).

1.3. Preparation of vegetable extracts

In order to obtain the plant extract, the method described by Shami et al., 2013; was followed with some modifications. Briefly, fourteen g samples of homogenized dried powder of grounded leaves and others with grounded stems, were added to 200 mL of absolute ethanol (Jasso de Rodriguez et al., 2015); and stirred in a stirring plate (Thermo Scientific CIMAREC model SP131325Q, USA) at room temperature for 3 days with the aid of a magnetic stirrer, in darkness. Then, the mixture was filtered through Whatman No. 1 filter paper. The solvent was removed by rotary evaporation (IKA-RV 10 digital V, USA Inc.) under reduced pressure at temperatures below 60°C (Martins et al., 2014), the remaining solvent was eliminated by placing the flask on drying oven at constant weight. The

extract was preserved in Eppendorf tubes and placed in a freezer at -10°C until it's used in the bioassays.

1.4. Preparation of secondary metabolites Bacillus subtilis

Fermentation was realized in Landy medium added with tryptophan. Landy medium was prepared (glutamic acid 5.0 g/L, KH_2PO_4 0.5 g/L, K_2HPO_4 0.5 g/L, $MgSO_4$.7 H2O 0.2 g/L, $MnSO_4$.H2O 0.01 g/L, NaCl 0.01 g/L, $FeSO_4$.7 H2O 0.01 g/L, $CuSO_4$.7 H2O 0.01 g/L, CaCl2.2 H2O 0.015 g/L and tryptophan). A solution of glucose microfiltrated was added to the medium for a final concentration of 1%. A preinoculum was realized by inoculation in 50 mL of Landy medium, incubated 48 h at 28 °C. From preinoculum were prepared the fermentation broths at a concentration of $1x10^8$ CFU/mL in Landy medium and shaked for 72 h, 120 rpm at 25°C. The fermentation broths were centrifuged at 12,500 rpm to get the supernatant for in vivo assay (Anguiano-Cabello et al., 2016)

1.5. Preparation of inoculum phytopathogenic bacteria

For *in vitro* and *in vivo* assays, bacterial suspensions of *C. michiganensis*, *R. solanacearum*, *X. axonopodis,* were prepared in nutrient broth medium on Appleton Woods Orbit 300, Labnetshaker (Kings Norton, Birmingham) incubator at 26°C ± 1°C for 24 h (Fatmi and Shaad, 2002). Suspensions were adjusted to concentration of approximately $1x10^8$ colony forming units (CFU) mL^{-1}, according to McFarland standards, which corresponds to a wavelength of 600 nm equal to 0.283 (A_{600nm}=0.283)(Fontenelle et al., 2011).

1.6. In vitro evaluation of IC 50 by plaque microdilution technique

For this assay, round shaped well bottom microplates (96-wells) (Ultra Cruz Plate, USA) were used. A sample of 100 µL of nutrient broth (BD bioxon), supplemented with2,3,5-triphenyltetrazolium chloride (TTC, tetrazolium red, Sigma T-8877, St. Louis, USA.) indicator at 0.01% (w/v),were added to each well except on wells of column one. On line A of microplate the formulated of plants extract

(P.E.) (615.36 mg/L of *L. graveolens*, 862.66 mg/L of *L. tridentate, 384.77* mg/L of *J. dioica*, and 297.27 mg/L of *C. illinoensis*) samples were resuspended in 95% water and ethanol, at an initial concentration at 100 %, were add 100 µl in wells of columns four of the microplate to carry out the serial dilution were serially diluted up to 50% with water in 96 well microtiter plates (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0.019 %). By line B were tasted the formulated with plants extract at 75% and 25% metabolites secondary (P.E.B.s.) (615.36 mg/L of *L. graveolens*, 862.66 mg/L of *L. tridentate, 384.77* mg/L of *J. dioica*, and 297.27 mg/L of *C. illinoensis* and metabolites of *Bacillus subtilis*) and in line C de Biobacter O® product was added as the same dilution on line A. Bacterial suspension (*C. michiganensis*, *R. solanacearum*, *X. axonopodis)*, previously prepared, were seeded in the wells of microplate at 100 µl except for the wells of the first column, bacteria concentration contained about $1x10^8$ colony forming units (CFU) CFU/ml. The third column of the microplates columns contains the medium, TTC, ethanol depending on the extract to be tested, as well as bacteria without any sample of plant extracts, in order that this well behave as the control of the evaluation. Immediately, the microplates were covered with its lid and incubated at 28°C overnight. The absorbance of bacteria inhibition microplate assay was read at 540 nm in a microplate reader (Thermo Scientific Multiskan GO, USA) controlled with Thermo Scientific Skan It software. The assay test was carried out in triplicates for each bacteria. The percent of bacteria inhibition (%) was calculated by the following equation:

$$\% \textit{ Inhibiton} = ((A_{control} - A_{sample}) / A_{control})*100$$

Where $A_{control}$ is the absorbance of the column three of all lines and A_{sample} is the rest absorbance of the samples from column four to twelve. The IC_{50} which is the concentration of the samples extracts

1.7. Material plant and conditions in greenhouse

Treatments with plants were formulated with seeds of tomato cultivar Saladette (*Lycopersicon esculentum*); obtained from the Nova-Gawepa© (France). Tomato seeds were surface-sterilized with a 2% sodium hypochlorite for 2 min. (Sleim et al., 2011), washed thoroughly with sterilized water and planted in to plant germination tray with peat moss (Premier© Sphagnum peat moss turbe of sphagnum, Canada). Plants were grown under controlled conditions (at 25 °C ± 5 with 65-80% RH and under 12000-14000 Lux from tungsten filament lamps for 16-h photoperiod) (Bastas, 2014). After 4 weeks, seedlings were transplanted into greenhouse black polypropylene bags (12 x 12 cm) containing sterilized experimental soil and peat moss (1:1) (Seleim et al., 2011); plant were watered by dripping with nutrient solution containing 175.1 g - KNO_3, 94.9 g - $Ca(NO_3)_2$, 14.8 g - $Mg(NO_3)_2$, 37.3 g - K_2SO_4, 20.2 ml - H_3PO_4, 76.7 ml - HNO_3 and 26.7 g - minor elements ULTRASOL® per 400 L.; three times per week for seven minutes. Plants were grown under natural photoperiod at 25 °C ± 5 with 65-80% RH. (Fontanelle et al., 2011).

1.8. Plant growth promotion and disease control assay

After one week of transplanted, stems were inoculated by 50 µL of the bacterial suspension (1.5), were injected in to 60 green house tomato plants (20 by *C.michiganensis*, *R. solanacearum*, and *X. axonopodis* respectively), at the second or third true-leaf sage as described by Fatmi et al., 2002. Tomato plants were treated with 8 mL ± 1 of sprinkler OSATU Tango 1.5 L (Spain) by drench soil, around of the root on plants; at first, twenty and forty day. The treatments (T) were defined as T1 – absolute witness (tomato plant without bacteria, only nutritional conditions); T2 – commercial control (tomato plant with bacteria and Biobacter O®); T3 – bacterial witness (tomato plant with bacteria); T4 – formulated of plants extracts (tomato plant with bacteria and formulated of *L. graveolens*, *L. tridentate, J. dioica*, and *C. illinoensis*); and T5 – formulated of plants extracts with metabolites of *B. subtilis* (tomato plant with bacteria and formulated of *L. graveolens*, *L. tridentate, J. dioica*, *C. illinoensis* and 25% of metabolites of *B. subtilis*).

1.9. Effects on agronomic parameters and percentage of severity

The plants were allowed to grow for 60 days (Fatmi et al., 2002) and then harvested. During the 60 days of the experiment were evaluated at 5 days interval; height of the plant and root, the width of the stem and the concentration of chlorophyll with SPAD 502DL Plus Minolta (Spectrum Technologies, Inc; IL, USA). Plants of different treatments were removed, washed with distilled water, blotted with tissue paper, and the dry weight of leaves, roots and stem were derminated. Plants were dried at 60° C for 72 h and dry weight was recorded, four replicates were used for the experiments (Abo-Elyusr and Asran, 2009). Data regarding the disease intensity were recorded starting from 10^{th} day to 50 days at 5 days interval according to scale used by Thahir et al., 2016. (0 = Free from infection; 1 = traces 25% leaf area killed; 2 = 26 – 50 % leaf area killed; 3 = 51 - 75 % leaf area killed and 4 = 76 – 100 % leaf area killed). Efficacy control was calculated using the following formula (Seleim et al., 2011):

*% Efficacy = (Disease incidence of control - disease incidence of treatment group)/(Disease incidence of control)*100*.

1.10. Data analysis

The experiment of % inhibition bacteria assays were established under completely randomized design with three repetitions and three treatments. A Probit analysis was performed to determine the 50% inhibitory concentration (IC_{50}) of each extract. The IC_{50} values were statistically calculated and associated with Chi-square values significant at $p<0.05$ level. The experiment of plant growth promotion and disease control assay agronomic parameters, percentage of severity were established under completely randomized design with four repetitions and five treatments. Analysis of variance (ANVA) and mean test were performed by Tukey test ($p=0.05$) using the SAS-PC System for Windows. Data were analyzed using SAS V 9.0. software for windows.

2. Results and discussion

2.1. Isolation and identification of bacteria

PCR detection in bacteria's suspensions confirmed the species being sought. By *C. michiganensis* the expected DNA product of 614 bp for Cmm5/Cmm6 primers. On other hand, *R. solanacearum*, displayed a figment of 504 bp for PEHA3/PEHA6 primers and finally *X. axonopodis,* showed a product of 425 bp for primers BSX1/BSX2.

2.2. In vitro evaluation of IC 50 by plaque microdilution technique

The antibacterial activity of Mexican desert plants, metabolites of *Bacillus subtilis* and Biobacter O® were evaluated against three phytopathogens causing damage in tomato crop. The antibacterial activity of the organic solvent extract showed varying magnitudes of inhibition patterns with commercial control depending on the susceptibility of the taste microorganism (Table 1). By *C. michiganensis* the best treatment was P.E.B.s. CI_{50} with 9.26% of the product; Lanna-Filho et al., 2013 demostrated that cell suspensions, supernatants and purified protein fractions of two endophytic *Bacillus* isolates were able to induce systemic resistance in tomato against *X. vesicatoria*. In other hand P.E showed the best CI_{50} with 2.12% against *R.solanacearum* and 2.49% against *X.axonopodis*. Sukanya et al., (2009) showed the ethnobotanical efficacy of Indian plants *Achyranthes aspera, Artemisia parviflora, Azadirachta indica, Calotropis gigantean, Lawsonia inermis, Mimosa pudica, Ixora coccinea, Parthenium hysterophorus and Chromolaena odorata*, against phytopathogenic bacteria (*X.vesicatoria* and *R. solanacearum*); leaves were extracted using different solvents such as methanol, ethanol, ethyl acetate and chloroform. Among treatments, maximum in vitro inhibition was scored in methanol extracts of *C. odorata* which inhibition against *E. coli, S. aureus, X. vesicatoria* and *R. solanacearum*. Biobacter O®, showed the best antibacterial activity against *C. michiganensis*. The formulates of desert Mexican plants showed the broad

spectrum of antibacterial activity on the tasted microorganism, that could be potential alternative to traditional chemical control of phytopathogenic bacteria on greenhouse conditions.

Table 1. Inhibition concentration IC_{50} (concentration of product expressed by percentage) to inhibit *C. michiganensis, R. solanacearum, and X. axonopodis,* by microdilution assay.

	C. michiganensis **CI_{50} (%)**	***R. solanacearum*** **CI_{50} (%)**	***X. axonopodis*** **CI_{50} (%)**
P.E	10.17±0.16	2.12±0.14	2.49±0.12
P.E.B.s	9.26±0.25	5.47±0.14	53.65±0.16
Biobacter O®	10.45±0.02	21.18±0.11	31.53±0.02

Data are means of three determinations.

2.3. Evaluation of agronomic parameters, percentage of incidence, severity and efficacy

The three products significantly inhibited the growth bacterial infection compared with untreated control. Results in Table 2 and Table 3 show that the three formulates increased the agronomic parameters. The best treatment against *C. michiganensis, R. solanacearum* and *X. axonopodis were* T5 in almost agronomic parameters. The results of this study are similar to Balestra et al., (2009) that showed the antibacterial effect of *Allium sativum* and *Ficus carica* extracts on tomato bacterial pathogens *Pseudomonas syringae pv. tomato* (Pst*), X. vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* reduced disease incidence by 58 and 30%, and disease severity by 68 and 22%, respectively.

Table 2. Evaluation of height stem and root, width stem and chlorophyll on greenhouse conditions at 50 day.

Bacteria	**Treatment**	**Height stem (cm)**	**Height root (cm)**	**Width stem (mm)**	**Chlorophyll (SPAD)**
C. michiganensis	**T1**	34.75 a	23.93 c	5.06 ab	35.85 a
	T2	26.45 ab	27.63 b	5.43 a	26.83 ab
	T3	19.63 b	23.63 c	4.38 b	17.88 b
	T4	26.75 ab	34.00 a	5.44 a	30.45 a
	T5	32.75 a	36.13 a	5.96 a	32.12 a
R. Solanacearum	**T1**	57.23 ab	26.13 b	4.59 b	36.03 a

	T2	47.88 bc	26.25 b	5.35 ab	22.45 bc
	T3	43.38 c	17.13 c	6.17 a	16.83 c
	T4	57.13 ab	35.75 a	5.46 ab	32.65 ab
	T5	61.50 a	37 a	5.24 ab	33.18 ab
	T1	53.75 a	12.25 b	6.09 ab	34.38 a
	T2	46.25 ab	15 b	6.05 ab	19.13 b
X. axonopodis	**T3**	40 b	6.25 c	5.04 b	13.43 b
	T4	50.75 a	24.75 a	6.23 ab	18.4 b
	T5	51.5 a	25.88 a	6.67 a	23.38 ab

Values with the same latter within each column are equal (Tukey, α=0.05%)

Table 3. Evaluation of weigth dry biomass of roots, stems and leaves on greenhouse conditions at 50 day.

Bacteria	Treatment	Weight dry biomass root (g)	Weight dry biomass stem and leaves (g)
	T1	0.87 a	2.64 a
	T2	0.62 ab	1.47 b
C. michiganensis	**T3**	0.47 b	2.17 b
	T4	0.99 a	2.49 ab
	T5	1.12 a	2.82 a
	T1	3.29 b	3.47 b
	T2	3.67 ab	4.22 a
R. Solanacearum	**T3**	3.42 b	3.42 b
	T4	4.34 a	4.54 a
	T5	4.47 a	4.34 a
	T1	2.85 cb	8.79 a
	T2	2.55 cb	7.07 ab
X. axonopodis	**T3**	1.88 c	4.64 b
	T4	3.63 b	7.77 ab
	T5	5.20 a	8.47 a

Values with the same latter within each column are equal (Tukey, α=0.05%)

The treatments two, four and five showed significant reductions in the severity (Table 4); of bacterial infection on greenhouse tomato crop. The most significant reductions of severity were conferred by T5 (75%) against *C. michiganensis,* T5 (50%) against *R. solanacearum* and T4 and T5 with 25% against *X. axonopodis*. However, different levels of protection were provided by the treatment composition. The differential ability of the treatments evaluated in this study to the distinct products ant with possible a phytochemical variability composition.

Table 4. Evaluation of percentage of incidence, severity and efficacy of treatments on greenhouse conditions at 50 day.

Bacteria	Treatment	Incidence (%)	Severity (%)	Efficacy (%)
C. michiganensis	**T1**	0 b	0 d	NA
	T2	100 a	50 b	0 c
	T3	100 a	100 a	0 c
	T4	50 ab	12.50 d	50 b
	T5	25 b	31.25 c	75 a
R. Solanacearum	**T1**	0 b	0 c	NA
	T2	100 a	100 a	0 c
	T3	100 a	100 a	0 c
	T4	75 a	37.5 b	25 b
	T5	50 ab	12.5 bc	50 a
X. axonopodis	**T1**	0 b	0 b	NA
	T2	100 a	100 a	0 b
	T3	100 a	100 a	0 b
	T4	75 a	25 b	25 a
	T5	75 a	25 b	25 a

Values with the same latter within each column are equal (Tukey, α=0.05%)

3. Conclusion

However, the importance of the obtained results must be analyzed with caution, since experiments conducted in greenhouses, described in the literature, often do not reflect the field situation. The bactericidal activity of these treatments gives new opportunities to improve control against different tomato bacterial diseases that cause losses. These treatments are also suggested for preventive control and reduces the severity diseases caused by the above pathogens. Studies on tomato crop fields are necessary to evaluate the efficacy of these natural substances on tomato plant development and tomato fruit production.

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Conclusiones Generales

El artículo de revisión registra resultados sobre el uso de extractos de plantas, así como de rizobacterias y hongos que promueven el crecimiento de plantas (con potenciales propiedades bioactivas, que exhiben actividad antimicrobiana, antioxidante y actividad de crecimiento significativa) que apoyan su uso en el tratamiento de algunas enfermedades fitopatógenas del tomate.

Los extractos acuosos y etanólicos de *Agave lechuguilla, Carya illinoinensis, Jatropha dioica, Larrea tridentata, Lippia graveolens, Origanum vulgare,* así como extractos de metabolitos secundarios *Bacillus spp.* exhibieron actividad antibacteriana contra *A. tumefacensis, C. michiganensis, P. syringae, R. solanacearum y X. axonopodis.*

Los metabolitos secundarios de *Trichoderma spp.* no presentaron actividad antibacteriana contra *A. tumefacensis, C. michiganensis, P. syringae, R. solanacearum y X. axonopodis.*

Hojas, tallos y raíz de *Agave lechuguilla, Carya illinoinensis, Jatropha dioica, Larrea tridentata, Lippia graveolens, Origanum vulgare* presentaron contenidos fenólicos y flavonoides y actividad antioxidante, así como una gran diversidad de componentes fitoquímicos, por su parte la espectroscopia IR demostró semejanza en compuestos observados en la literatura, con posible actividad antibacteriana.

Los bioformulados generados, se pueden emplear como control y prevención; ya que reducen la incidencia y severidad de las enfermedades causadas por *A. tumefacensis, C. michiganensis, P. syringae, R. solanacearum y X. axonopodis.*