UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS, INGENIERÍA Y MEDICINA

PROGRAMA MULTIDISCIPLINARIO DE POSGRADO EN CIENCIAS AMBIENTALES

TESIS QUE PARA OBTENER EL GRADO DE

DOCTORADO EN CIENCIAS AMBIENTALES

DIAGNÓSTICO Y CARACTERIZACIÓN DE LA APICULTURA Y VALORACIÓN BIOLÓGICA DE *Larrea tridentata* COMO AGENTE ACARICIDA EN SAN LUIS POTOSÍ

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AGOSTO 2023

CRÉDITOS INSTITUCIONALES

PROYECTO REALIZADO EN:

Instituto de Investigación de Zonas Desérticas, UASLP

El Mezquite, Villa de Arista, S.L.P.

El Mezquital, Villa de Arriaga, S.L.P.

San Elías, Armadillo de los Infante, S.L.P.

CON FINANCIAMIENTO DE:

Proinnova-CONACYT

México-Nutrición y Genética Saludable

Acuerdo numero 251911

A TRAVÉS DEL PROYECTO DENOMINADO:

Escalamiento a planta piloto de aditivo alimentario multifuncional de origen nanoarcilloso y botánico para emplearlo en el área de nutrición y salud animal. Convocatoria del programa de estímulos a la innovación del Proinnova-Conacyt. empresa; Nutrición y Genética Saludable S.A. de C.V. registro 251911.

AGRADEZCO A CONACyT EL OTORGAMIENTO DE LA BECA-TESIS

Becario No. 416684

EL DOCTORADO EN CIENCIAS AMBIENTALES RECIBE APOYO ATRAVÉS

DEL PROGRAMA NACIONAL DE POSGRADOS DE CALIDAD (PNPC)

AGRADECIMIENTOS

A instituciones

Agradezco el apoyo brindado por el personal del Instituto de Investigación de Zonas Desérticas de la Universidad Autónoma de San Luis Potosí.

Al Programa Multidisciplinario de Posgrado en Ciencias Ambientales.

A personas

Agradezco a mi director de tesis el Dr. Juan Carlos García López por su constante apoyo, confianza y motivación.

A mis asesores, el Dr. Gregorio Álvarez Fuentes y Dr. Carlos Contreras Servín.

A los apicultores del estado de San Luis Potosí por su disposición y amabilidad al compartir sus conocimientos y experiencias.

A mi familia

Por su eterno apoyo, consejos y cariño. Gracias por estar cada vez que los he necesitado.

A Rebeca

Por atreverte a incursionar en el mundo de la apicultura.

DEDICATORIA

Para Valentina

Futura apicultora

Porque le encanta la miel y no teme a las abejas

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RESUMEN

La gobernadora (*Larrea tridentata*) es un arbusto perene ampliamente distribuido en el norte de México y ha sido históricamente utilizado por los habitantes de la región debido a sus propiedades medicinales, esto ha conducido a que durante más de setenta años desarrollara investigación con el objetivo de caracterizar sus propiedades y usos potenciales, entre los que destacan sus efectos bactericidas, fungicidas, nematicidas, viricidas, antioxidantes y recientemente anti-cancerigenos. Dichos efectos pueden ser atribuidos a la diversidad de metabolitos que se encuentran presentes principalmente en las hojas, especialmente el lignano ácido nordihidroguayaretico (ANDG) que destaca con el metabolito mayoritario y uno de los más estudiados. Poco se ha explorado sobre el potencial que tiene la gobernadora para ser utilizada dentro del campo de la sanidad animal. Al respecto, se han documentado efectos positivos en las variables productivas de ganado bovino, así como efectos bactericidas a nivel intestinal en pollos de engorda.

La apicultura, es una actividad pecuaria que demanda la utilización de productos naturales para la atención de problemas sanitarios como la varroa; ya que dentro de la región altiplano, centro y media del estado de San Luis Potosí, dicha parasitosis presenta una prevalencia del 100%, así como un porcentaje promedio de infestación de 3.8%, destacando el químico sintético amitraz como el producto utilizado por el 59% de los apicultores para el control de varroa, mientras que el 34% muestra interés por uso de alternativas orgánicas, de manera que la gobernadora puede resultar una opción viable para el manejo y control de varroa, ya que de acuerdo con los resultado obtenidos en el presente estudio, la aplicación de extractos acuosos y etanolicos puede llegar a alcanzar porcentajes de mortalidad de varroa que oscilan entre el 51 y 53% a nivel *in vitro*, así como 49 a 72% al ser aplicados directamente en la colmena. Aun cuando estos resultados no son equiparables con los obtenidos por químicos sintéticos especialmente diseñados para tal objetivo, se deben considerar los beneficios de utilizar compuestos naturales que representen una alternativa segura para el control de varroa, y que pueden ser incorporados en programas integrales de manejo del ácaro, tomando en cuenta también los diferentes factores ambientales y de manejo que pueden influir o estar relacionados con el desarrollo de las poblaciones de varroa. Cuya variación debe ser altamente significativa, ya que como se muestra en el presente trabajo, el comportamiento de la población de varroa no presento diferencias significativas durante dos temporadas de floración en la misma región apícola, aun a pesar de la variación en la fortaleza de las colonias de *A. mellifera*. Así como tampoco se encontró diferencia entre los porcentajes de infestación de varroa de la zona altiplano, centro y media del San Luis Potosí, a pesar de sus diferencias climáticas.

Palabras clave: *Apis mellifera, Varroa destructor, Larrea tridentata*, potencial acaricida, factores ambientales.

ABSTRACT

The Creosote bush (*Larrea tridentata*) is a perennial shrub widely distributed in northern of Mexico and has historically been used by the habitants of the region due to its medicinal properties, this has led to the development of research for more than seventy years with the objective of to characterize its properties and potential uses, like its bactericidal, fungicidal, nematicidal, virucidal, antioxidant and recently anti-carcinogenic effects. These effects can be attributed to the diversity of metabolites that are present mainly in the leaves, especially the lignan nordihydroguayaretic acid (NDGA) that stands out as the major metabolite and one of the most studied. Little has been explored about the potential of the Creosote bush to be used within the field of animal health. Positive effects have been documented on the productive variables of cattle, as well as bactericidal effects at the intestinal level in broilers.

Beekeeping is a livestock activity that demands the use of natural products for the control of health problems such as *Varroa destructor*. In the altiplano, center and middle region of the state of San Luis Potosí, this parasitosis presents a prevalence of 100%, as well as an average infestation percentage of 3.8%, highlighting the synthetic chemical amitraz as the product used by 59 % of beekeepers for the control of varroa, while 34% show interest in the use of organic alternatives, so that the Creosote bush can be a viable option for the management and control of varroa, since according to the results obtained in In the present study, the application of aqueous and ethanolic extracts can reach percentages of varroa mortality between 51 and 53% at the *in vitro* level, as well as 49 to 72% when applied directly in the hive. Even when these results are not comparable to those obtained by synthetic chemicals specially designed for this purpose, the benefits of using natural compounds that represent a safe alternative for varroa control should be considered, and that can be incorporated into integrated management programs of *V. destructor*, also considering the different environmental and management factors that may influence or be related to the development of varroa populations. Whose variation must be highly significant, since as shown in the present work, the behavior of the varroa population did not present significant differences during two flowering seasons in the same beekeeping region, even despite the variation in the strength of the colonies of *A. mellifera*. Likewise, no difference was found between the percentages of varroa infestation in the altiplano, central and middle region of San Luis Potosí, despite their climatic differences.

Key words: *Apis mellifera, Varroa destructor, Larrea tridentata*, acaricidal potential, environmental factors.

INTRODUCCIÓN

La abeja mellifera, empleada en la apicultura, es una especie de gran relevancia a nivel mundial especialmente por su actividad polinizadora en países como Italia, Grecia, España, Francia, Alemania, Suiza, Austria, Estados Unidos y Países Bajos (Tirado *et al.*, 2013). La apicultura, es una actividad productiva que complementa a la agricultura, y ambas desempeñan un papel fundamental en la conservación de la biodiversidad y la producción de alimentos, ya que la tercera parte de la producción agrícola depende de la polinización a través de insectos como las abejas. La apicultura en México, en especial en las regiones tropicales, es una actividad que se practica desde hace varias centurias (Magaña-Magaña *et al.*, 2016). Actualmente, además de su importancia ecológica, la apicultura ha adquirido gran relevancia socioeconómica, ya que representa una fuente importante de empleos e ingresos en el medio rural derivados de la producción de miel principalmente (Magaña Magaña *et al.*, 2007). Durante el 2022 el valor de las exportaciones de miel natural alcanzo los 61 millones de dólares (Secretaría de Economía, 2023) y se generaron alrededor de 100 mil empleos directos. En 2021, México ocupo el octavo lugar en producción de miel en el mundo con un estimado de 62,080 toneladas, y el noveno lugar como exportador (Orús, 2023). En San Luis Potosí se estimo una producción de 1,145 ton de miel durante el 2020, ocupando el decimo cuarto lugar a nivel nacional (SIAP, 2023) gracias a la labor de 921 apicultores que manejan a diario alrededor de 38, 886 colmenas (López, 2016). El estado de San Luis Potosí se encuentra en una ubicación privilegiada, ya que en el convergen tres de las cinco regiones apícolas del país, lo que le brinda la posibilidad de producir una amplia variedad de mieles de diferente origen floral, siendo la zona Altiplano, Centro y Media de San Luis Potosí, áreas importantes para el desarrollo de la apicultura por su producción de miel de mezquite, azahar y multiflora, las cuales son muy valoradas en el mercado europeo.

A partir de 1998, apicultores de diferentes partes del mundo, principalmente de Francia, Bélgica, Suiza, Alemania, Holanda, Italia, España, Reino Unido y Norte América informaron sobre un inusual fenómeno de mortalidad y debilitamiento de las colonias de *Apis mellifera* (Neumann y Carreck, 2010). La creciente problemática de pérdida de colonias de abejas a nivel mundial, denominada “Síndrome de Colapso de las Colonias” (CCD por sus siglas en inglés), ha despertado un gran interés por el estudio de los factores involucrados en dicho fenómeno, entre los que destaca la presencia de patógenos, cambio climático, nutrición, plaguicidas y organismos genéticamente modificados (UNAM y SENASICA, 2017).

En México, la mayor inquietud de los apicultores se centra en la tendencia a la baja de la producción de miel y la pérdida de colonias de abejas, mismas que pudieran estar vinculadas a la presencia de enfermedades parasitarias, principalmente a la llegada del parásito *Varroa destructor*, a mediados de la década de los años 90, y asociada a un 55% de las unidades de producción apícola (UPA) como una de las principales causas de mortandad. Por otra parte, aspectos ambientales como variación de las temporadas de floración, sequía, aumento de la temperatura, retraso en la temporada de lluvias y menor intensidad de estas, también son

referidas como causas de mortandad en 60.6% de las UPA. Adicionalmente, las regiones apícolas del Norte y Golfo tienen algunos problemas más que destacar, al ser las regiones del país más expuestas a los insumos fitosanitarios (UNAM y SENASICA, 2017) presentan un incremento en los casos de envenenamiento de abejas; además, la exposición a los pesticidas durante el otoño puede inmunosuprimir la colmena y favorecer el desarrollo del parasito *V. destructor* y la enfermedad generada por *Nosema* spp., disminuyendo el crecimiento de la colmena durante y después del invierno.

Hablando específicamente de la varroasis, se ha documentado como la principal causa de muerte de colonias de abejas durante el invierno en Canadá, ya que está asociada a más del 85% de los casos de mortalidad de éstas (Guzmán-Novoa *et al.*, 2010). De igual forma, su efecto detrimental sobre la producción de miel, se ha observado en algunos estudios, presentando correlaciones negativas de r= -0.44 entre el nivel de infestación y la producción de miel (Medina-Flores *et al.,* 2011). En un estudio realizado en Valle de Bravo, Estado de México se analizó el impacto de la varroasis sobre la producción de miel, encontrando que colonias de abejas tratadas con un acaricida contra varroa produjeron 65% más miel que colonias no tratadas (Arechavaleta-Velasco y Guzmán Novoa, 2000). Esto se debe posiblemente a una reducción en el periodo de vida de las abejas, ya que una abeja parasitada reduce aproximadamente el 50% de su tiempo de vida en comparación con una abeja sana (De Jong y De Jong, 1983), lo cual provoca una reducción en la población productora de miel. Jean Prost y Le Conte (2007) afirma que, no se sabe cómo afecta el clima, flora, y las prácticas apícolas, a la biología de la varroa; por lo cual, se requieren más estudios que establezcan la relación parásito-producción de miel en muchas regiones del país, pues tanto las condiciones ambientales como el tipo de abeja, pueden influir en el efecto de la varroasis sobre la fisiología y productividad de las abejas (Murilhas, 2002).

El control de la varroa comúnmente se ha realizado utilizando tratamientos químicos, especialmente compuestos sintéticos, debido a su fácil aplicación, bajo costo y por lo general alta eficacia. Entre la gama de opciones químico-sintéticas mundialmente utilizadas encontramos la formamidina Amitraz, el organofosforado cumafós, así como los piretroides fluvalinato y flumetrina (Cameron y Ellis, 2021). El uso indiscriminado de estos productos ha favorecido la aparición de resistencia en muchos países (Thompson *et al.*, 2002) y disminución en su eficacia, como en el caso del fluvalinato y cumafós cuya eficacia documentada por diferentes autores no logra superar el 24% (Mozes-Koch *et al.,* 2000; Thompson *et al*., 2002, Maggi *et al*., 2009; Haber *et al*., 2019). Por el contrario, compuestos como el amitraz presentan porcentajes de eficacia superiores al 76%, aun cuando también se ha demostrado algún nivel de resistencia al producto (Al Naggar *et al*., 2015; Gregorc *et al.,* 2018; Jack *et al.,* 2020). México corre el mismo riesgo, pues solo cuenta con dos piretroides recomendados para dicha finalidad; fluvalinato y flumetrina (González-Gómez *et al.*, 2006). Además, es necesario considerar que los acaricidas químicos al entrar en contacto con la cera y con el pelo de las abejas, se disuelven en la grasa de la cera; almacenándose en esta y pasando de ahí a la grasa del polen, mismo que posteriormente será consumido por abejas adultas, crías o será mezclado con la miel, afectando directamente la salud

de la colonia y contaminando los productos de esta. En este sentido, se ha observado una disminución de entre el 10 y 30% en la longevidad de las abejas criadas en ceras con residuos de acaricidas como clorfenvinfos y coumafós. Además, existen efectos colaterales al presentarse dosis sub-letales, que, aunque no provocan mortandad aguda, a largo plazo provocan la inhibición de la producción de péptidos antimicrobianos, afectando el sistema inmunológico de la abeja.

No existen tratamientos químicos sintéticos cuya efectividad sea del 100%, por lo cual, aquellos individuos que logran sobrevivir debido a su mayor resistencia darán origen a la siguiente generación, y al paso del tiempo esto se traducirá en una población considerablemente resistente, motivando a un escalamiento en las dosis de aplicación y residualidad de los acaricidas en la colmena. Dichos residuos pueden llegar a incrementar su toxicidad al ser combinados con agroquímicos presentes en el polen (Chauzat *et al*., 2006). Este sinergismo letal entre pesticidas y acaricidas puede ocurrir al acumularse en las matrices de la colonia a lo largo del tiempo, por lo que este efecto residual es más importante ahora que hace 20 años (Le Conte *et al.* 2010). Los compuestos naturales hasta el momento no han mostrado esta particularidad. Por lo cual, existe la necesidad de investigar nuevas alternativas menos contaminantes para el control de *V. destructor*, que tengan el potencial para ser alternados con los tratamientos químicos o que puedan reemplazarlos en su totalidad. Aspecto de vital importancia hoy en día, para mercados internacionales con elevados estándares de calidad e inocuidad.

Los productos orgánicos para el manejo de plagas tienen gran importancia en la actualidad, ya que muchos apicultores se han opuesto al uso de químicos sintéticos en sus colmenas por considerarlos demasiado agresivos con las abejas y poco seguros de utilizar. Por otra parte, el elevado porcentaje de la producción nacional destinada a la exportación demanda el cumplimiento de la normativa que permite satisfacer los requerimientos del mercado extranjero, además del gran potencial que existe en la diferenciación de productos como la miel a través de certificaciones orgánicas o sellos verdes. Murillo y Velandia (2014) mencionan que acciones como la caracterización del origen botánico de la miel, certificaciones orgánicas y kosher pueden ser una buena estrategia para brindar un valor agregado a la miel dentro del mercado estadounidense. De acuerdo con el SENASICA (2022), aproximadamente el 6.5% de los productores apícolas en México se encuentran certificados bajo la Ley de Productos Orgánicos, gracias a los cuales el país ocupa el décimo lugar en distribución de miel orgánica en el mundo. Dentro de los lineamientos de la apicultura orgánica 2022, se permite el uso de compuestos orgánicos como el ácido fórmico, láctico, acético y oxálico para el control de *V. destructor*, al igual que otros productos como el timol, mentol, eucalipto, alcanfor y extractos de plantas de acuerdo con la Lista Nacional de sustancia permitidas para la operación orgánica agropecuaria, descartando por completo el uso de acaricidas químicos sintéticos.

Entre los compuestos orgánicos más populares y que ha demostrado mayor efectividad en el control de varroa encontramos ácidos orgánicos como oxálico y fórmico, al igual que aceites esenciales como el timol; mismos que debido a su naturaleza presentan una eficacia muy variable

(Cameron y Ellis, 2021). En el caso de ácido fórmico se ha observado una eficacia del 35 al 75% (Underwood y Currie, 2003), aun cuando su mecanismo de acción no está claramente dilucidado, se sabe que inhibe el transporte de electrones a nivel mitocondrial en la varroa al unirse con el citocromo C oxidasa. Hasta el momento ha demostrado ser el único acaricida con efecto sobre la varroa tanto en fase foretica como reproductiva (Fries, 1991), pero con riesgo de generar mortalidad en la cría de las abejas y reinas en ambientes con temperaturas elevadas (Giovenazzo y Dubreuil, 2011). Por su parte, el ácido oxálico ha llegado a presentar porcentajes de mortalidad de varroa cercanos al 100% (Gregorc y Sampson, 2019), especialmente en temporadas de baja disponibilidad de cría de abejas, ya que actúa por medio del contacto directo con el ácaro, favoreciendo además el desprendimiento del cuerpo de la abeja e incrementando el acicalamiento o grooming (Schneider *et al*., 2012). Los métodos más comunes de aplicación son el rociado en los bordes superiores de los bastidores, la aspersión directamente a las abejas (Rademacher y Harz, 2006), la disolución en el jarabe durante periodos de alimentación artificial, así como la colocación de cristales para sublimado (Cameron y Ellis, 2021). Respecto a los aceites esenciales, el timol es el compuesto comúnmente utilizado en la apicultura para el control de varroa y que se encuentra disponible en formulaciones comerciales. Se estima que su eficacia varia del 50 al 80% (Coffey y Breen, 2013), y depende en gran medida de la temperatura y la cantidad de cría de abeja en la colmena (Calderone, 1999), llegando a presentar un ligero riesgo para la cría y la reina cuando se aplica en periodos de elevada temperatura (Floris *et al*., 2004). Al igual que otros aceites esenciales su principal compuesto son los monoterpenos, los cuales actúan como fumigante frente a varroa (Imdorf *et al*., 1999). Si bien existe una gran cantidad de aceites esenciales como el ajo, mentol, trébol, orégano, romero, neem, entre otros; que han demostrado poseer propiedades acaricidas, la falta de eficiencia en los métodos de extracción, así como las formulaciones con concentraciones muy variables representan un importante obstáculo al intentar alcanzar cifras consistentes en el control de varroa (Sabahi *et al*., 2017).

La Gobernadora (*Larrea tridentata)* es una notable fuente de productos naturales, siendo los lignanos fenólicos el grupo más importante entre sus metabolitos secundarios debido a sus propiedades antioxidantes y biocidas; específicamente el ácido nordihidroguaiarético (NDGA), que ha sido catalogado como uno de los lignanos mayoritarios y más representativo de esta planta (Gnabre *et al.*, 2015). El uso potencial de algunos lignanos ha sido estudiado anteriormente, obteniendo resultados comparables con la actividad de piretroides sintéticos comerciales, atribuyendo dicha cualidad a su capacidad inhibidora de sistemas enzimáticos (Taniguchi *et al.*, 1989). Por otra parte, Viglianco *et al.* (2006) considera que la gobernadora puede llegar a poseer importantes efectos repelentes y anti-alimentarios, representando una alternativa viable, no contaminante y aplicable a la producción agropecuaria, ya que el uso de compuestos naturales para la atención de problemas sanitarios como la varroa se debe considerar no solo como una opción en respuesta a problemáticas como la resistencia de los individuos frente al uso de químicos sintéticos, sino como un compromiso ambiental y contribución a la seguridad alimentaria global (Rodríguez y Niemeyer, 2005).

Por lo anterior, el objetivo del presente trabajo de tesis es presentar una revisión detallada de las propiedades y usos de la gobernadora que han sido documentadas has el momento, analizar el rol de los factores asociados a la fortaleza de colonias de *Apis mellifera* y la forma en que interactúan con el ácaro *Varroa destructor*; y por último evaluar la eficacia de los extractos de *L. tridentata* en el control de *V. destructor*.

OBJETIVOS

Determinar el porcentaje de infestación y prevalencia de *Varroa destructor* en colonias de *Apis mellifera* en la región altiplano, centro y media del estado de San Luis Potosí, así como la relación de dicha parasitosis con variables geográficas y ambientales

Analizar el rol de los factores asociados con la fortaleza de la colonia que pueden afectar la interacción *A. mellifera – V. destructor* durante dos temporadas de floración.

Documentar las propiedades y usos de la gobernadora (*Larrea tridentata*) al igual que su potencial para ser utilizada en el área de sanidad animal.

Evaluar la eficacia de extractos de *Larrea tridentata* para el control de *Varroa destructor* en colonias de *Apis mellifera*.

1. CREOSOTE BUSH (*Larrea tridentata*) PHYTOCHEMICAL TRAITS AND ITS DIFFERENT USES: A REVIEW

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1.1 ABSTRACT

Creosote bush (*Larrea tridentata*) is a perennial shrub present in Chihuahuan, Sonoran and Mojave deserts it contains diverse metabolites; among them lignans are the most important, one of the most studied is nordihydroguaiaretic acid (NDGA), this shrub has been studied for more than seventy years due to its great variety uses. The bactericidal effect of creosote bush has been well documented, as the fungicide, nematicidal, protozoa and viral effect. It has been used as an antioxidant to preserve meat in canned food. Recently research has been done on NDGA effects on anti-carcinogenic cells. There is scarce information about the use of creosote bush in livestock production. Some studies in sheep and broilers are available. The results of these research indicate that creosote bush could be used to improve productive variables in livestock and have an intestinal effect on bacteria.

Key words: *Larrea tridentata,* nordihydroguaiaretic acid, animal production.

1.2 BOTANICAL DESCRIPTION

Larrea tridentata commonly known as creosote bush (Figure 1), it´s a perennial shrub that belongs to the Zygophyllaceae family; its generic name *Larrea* was given in honor to the spanish scientific J.A. Hernández Pérez de Larrea, since he was the first one to describe it [1,2]. It´s a 3-10 ft evergreen woody plant, branched from its base with small leaves, opposed, brilliant, dark to yellowish-green with a lining of resin secrete by glandular trichomes during its development [3]. The flowers from 5 petal and yellow in color, grow lonely near the termination of young sprouts, they appear normally at the end of the winter or early spring, but they can bloom at any moment after a rain episode [4]. The fruit is a round capsule covered by a thick white villosity concentration [3] and contains five seeds that spread in spring and at the beginning of fall with the wind and rain [4]. Identified by the penetrating aroma and bitter flavor.

Figure 1.1 Creosote bush (*Larrea tridentata*) specimen.

Since it´s a perennial shrub it keeps the majority of leaves at low temperature and during drought. However, extreme periodic freeze may contribute to limit the actual distribution, because when the plant freeze xylem cavitation and embolism is induced [5]. Its wide distribution in areas considered unproductive, has generated studies about its commercial value, estimating 1 million ton of dry fodder and 100,000 ton of resin [6]. However, its fodder value is questionable, since its not palatable for livestock [7].

1.3 DISTRIBUTION

Creosote bush (*Larrea tridentata*) also known as hediondilla, gobernadora, guamis or greasewood [2] is the most characteristic species of the hot desert of North America. Its abundance has been increased in response to disturbance and shepherding [8]. Creosote bush is an abundant resource in the desert zones of Mexico, its found in Sonoran and Chihuahuan Desert, the states of North Baja California, South Baja California, San Luis Potosí, Coahuila, Durango, Zacatecas and Nuevo León [9], about 25% of the desert areas its covered with creosote bush [10]. In the United States of America *L. tridentata* represents about 17.5 million of Ha including the following states: Arizona, California, Nevada, Texas and New Mexico [9]. In South America specially Argentina and Bolivia, similar species to *L. tridentata* have been found. It has a wide range adaptation from sea level in Death Valley California, through more than 2,500 meter above sea level in the North Mountains of Mexico [11]. Creosote bush not only thrives in areas with abundant predominant vegetation, but also is genetically adapted, its genotype varies according to the region where its found, having two sets of chromosomes the species that grow in South America and the Chihuahuan Desert, and four sets of chromosomes those that grow in the Sonoran Desert, where the winter is mild and rain is present during summer and winter. In contrast, the plants that grow in the Mojave Desert are hexaploid (six sets of chromosomes), where they have a long and hot

summer [1,12]. It´s unknown if these chromosome variations respond to the region´s weather, or if they are correlated with the amount and diversity of secondary metabolite production [13].

1.4 ETHNOBOTANY

Creosote bush has been used as folk medicine for many years by North and South America tribes [14], for the treatment of different illnesses, commonly as aqueous or ethanolic extract, in poultice using leaves and tender stems. The native healer of Southwest USA, used creosote bush leaves infusion, known as "chaparral tea" [2]. Among the main uses is for stomach pain treatment, diarrhea, and ulcers [15]. Also, for treatment of venereal diseases [14], infertillity, menstruation pain, and postnatal inflammation [15,16] and as contraceptive [15]; also, in urinary tract infections [14] and cystitis [17]. The Pima Indians of Southeast of USA and Mexico used creosote bush for diabetes treatment [18]. It has anti-inflammatory and analgesic properties, and its used for neuritis and sciatica treatment [14], tooth pain [19], headache [15] breast cancer [20], allergies [21] and for liver diseases treatment [22]. The chaparral tea has been used for cold, influenza and cough treatment [15.19]. The Cahuilla tribe in the USA Southwest, used the stem and leaves infusion for cancer treatment [23]. It is considered one of the best antibiotic based in plants, because it´s effective against bacteria, virus, and internal and external parasite [24]; in addition its fungicide activity tested with Aspergillus, Penicillium and Fusarium [25].

1.5 PHYTOCHEMISTRY

Larrea tridentata is a valuable source of secondary metabolite, considering that approximately 50% of their dry weight of leaves is extractable material [26]; being the resin, the main reservoir of metabolite such as saponins, sapogenins, tannins, sterols, monoterpenes, sesquiterpenes, flavonoid glycoside (19 aglycone flavonoids), a large amount of essential oils (approximately 300 volatile compounds) and 67 non-volatile compounds [2,13].

Phenolic lignans are one of the most important metabolites in *Larrea* species [13], being the most prominent in relation to dry weight, followed by sponins, flavonoids, aminoacids and minerals [27]. In terms of natural chemical products, creosote bush is well known for its large amount of the acid lignan; nordihydroguaiaretic acid (NDGA) its well known as a powerful antioxidant [26,28]. It is well documented that this acid has antioxidant properties, anti-inflammatory, cytotoxic, antimicrobial and enzyme inhibitor [21,29,30]. The NDGA constitutes the 80% of all resin phenols [2] and 40 to 50% of total resin [31,32], while the resin constitutes 5 to 15% of leaves dry weight [2,31].

1.5.1 Secondary metabolites function of Larrea tridentata

The vegetal extracts of *Larrea* keep a wide range of pharmacological effects, this is a natural defense mechanism against threats such as microorganisms, insects, and other predatory animals [33]. Among these effects *L. tridentata* has anti-inflammatory, antioxidant, and anticancer properties [34]; also, it has a biocide activity against bacteria [35], fungus, virus, protozoa, insects and plants [36].

The resin keeps a wide range of compounds with the purpose of make the fodder less palatable, probably caused by the tannins [37], in addition it works as a antiperspirant, it forms a barrier on the surface of the leaf that decreases the transpiration more than the rate of CO_2 assimilation [38], and protects from UV radiation; these traits are very important for the success of species in desert environments [29]. In addition, the phenolic biopolymers and NDGA present in the resin, stem and leaves serve as a chemical defense from animal predators [29,39,40,41].

1.5.2 Phenolic lignans

Lignans are natural compounds elaborated by the plant for purpose of defense and can be found in a great variety of plants and in each part of its anatomy. Its structural units are synthesized through shikimic acid pathway by the bond of two phenylpropane units linked by the central carbons of their sided chains (link C_8-$C_{8'}$). The lignans were first defined as a phenylpropane dimers (C6-C3) by Haworth in 1942, who introduced the term "lignane" and subsequently change to "lignan" [42].

The lignan word is used in general, however there are different kind of lignans, among them are: lignans (contents two units C_6-C_3 linked by a inlace between subunits (C_8-$C_{8'}$), neolignans (contents two units C_6-C_3 but with a different inlace) and hybrid lignans or mixed heterodimers resulted from the coupling of monolignols or, in some cases, lignans or neolignans, with other substances like flavonoids, coumarins, xanthones, etc., that give origin to flavanolignans, cumarinolignans, estilbeno lignans, xanthone lignans, etc. [42].

In a particular way, lignans are subdivided in two groups, simple lignans and cyclo lignans; lineal type lignans were the first documented a few decades ago in desert zone plants [29]. Later, the cyclolignans and furanoid lignans were added to the list [26]. The first one represents only the link carbon-carbon through the positions 8 and 8´ of their sided chains. If oxygen is incorporated or not to the sided chain and the way, it does it can be establish four subtypes of simple lignans: Dibenci bhutan; Dibencilbuthirolactone: lignan lids; Tetrahydrofurans: epoxi lignan; Furofuranes: bisepoxi lignans.

The cyclo lignans result from another additional link carbon-carbon, which results in a new ring, and 4 subtypes arise: Ariltetrahydro-naftalen, Arildihydro-naftalen, Arilnaftalen and Dibencilcyclooctadiens [42,43]. In the plant, lingan physiological functions are linked to defense activities such as fungicide, bactericide, antioxidant, and enzyme inhibitors, it can be observed its activity in some woody species where these compounds give durability, quality, color, and texture to the wood [42]. In human medicine, lignans have showed pharmacological, antioxidant, anti-herpes, fungicide, and anti-inflammatory activities for more than 70 years; and recently has been increased the studies of this compound against VIH, papilloma human virus and breast cancer [44].

1.6 NORDIHYDROGUAIARETIC ACID (NDGA)

1.6.1 Human medicine applications

The use of plants for medicinal purposes has been linked to human race history for a long time ago, the record shows that old cultures like egyptians, romans, greeks, chinese and babylonian used this natural resource. It´s well known that at least 2% of the botanical resources have been exploded for the extraction of bioactive molecules, and approximately 80% of the world population recur to the use of herbal medicine for primary health attention [45].

It´s known that differrent vegetal extracts possess diverse pharmacological effects, showing anti-inflammatory and antioxidant properties [34], biological activities against a wide range of microorganisms [36], and chemotherapy due to its phytochemical content and low toxic effect [46].

In particular, medicinal lignan awoke the interest of the scientific community a few decades ago, thanks to the discovery of compounds like podophyllin known for being highly cytotoxic extracted from the roots of species of Podophyllum [47]. The NDGA inhibits the multiple cell culture cancer proliferation that include lung cancer [48], prostate [49], gastric [50], neuroblastom [51] breast cancer [20], hepatocellular human carcinoma [52].

1.6.2 Antioxidant properties

The lignan NDGA, has been used since the late 50´s in aging and longevity studies, because of this it has been the antioxidant no nutritious more used in experimental gerontology [53]. Until this moment, the studies involving NDGA and other antioxidants were performed basically in rodents, fruit flies and mosquitos (laboratory animals); this situation did not allow to test the whole antioxidant effects, since they were limited by laboratory conditions. Inadvertently, the application of antioxidant therapies, at that time unexplored, it occurred with livestock fed with creosote bush leaves that showed a high protein content compared to alfalfa, they were economic important for the livestock feeding in rangeland areas in the american southwest [6]. Moreover, since its a perennial bush, it kept a constant level of NDGA in the cattle´s diet during the whole year without variation on the different seasons [54].

1.6.3 Fungicidal properties

Recently, the resistance to several commercial fungicide products has gained interest for the use of natural original compounds, as an alternative for the control of fungi diseases since some of them inhibit fungi growth and have demonstrated to be effective on *in vitro* and *in vivo* conditions [55]. Particularly the creosote bush has been studied for its antifungic action, on several occasions under *in vitro* conditions at least 17 phytophatogenic fungi of economical importance have been tested, on *in vivo* studies as ground vegetative material or as an extract incorporated to the soil to control 6 fungi in agriculture crops [56,57,58].

Takemoto et al. (1975) [59] mention that the antifungal activity of lignans its attributed, in part, to the inhibition of extracellular fungal enzymes: cellulase, polygalacturonase, aril-β-glucosidase and lactase. In addition, Vargas et al. (2009) [60] have observed that the β-1,3-glucanase enzyme plays an important physiological function in the fungi morphogenetic process, in β-glucane mobilization, spore cellular division and germination [61], and is involved in the interaction plant-fungi-pathogen during its attack. Vargas et al. (2009) [60] observed that *L. tridentata* extracted with dichloromethane (3 mg/ml) is capable to inhibit in 96.5% the β-(1,3)-glucanase enzyme activity; in contrast to methanol raw extract of *L. tridentata* leaves that did not show any inhibitor activity of the enzyme. When the inhibitory activity of pure lignans was performed, it was observed that NDGA (10µM) is capable to inhibit 100% of the β-(1,3)-glucanase activity, while the lignan methyl-NDGA showed only 40% of inhibition. Regarding these results, it is believed that the inhibitory activity of NDGA can be related with the hydroxyil groups present in its structure, that can join to the catalytic site of β-(1,3)-glucanase and inhibit the enzyme activity as a result of that interaction. The enzymatic activation requires two functional carboxylic acids in the catalysis. The first corresponds to the general acid in the 175 glutamate, and the second to 170 glutamate, the catalytic residual involved in the nucleophilic attack over the substrate [62]. These residuals are completely kept in all β-glucanases available sequences [63], which means that the active site and the catalytic mechanism are similar inside of one family. Thus, 170 glutamate, in one polarity side from the chains of glucanase catalytic site, could be the main force of bond between the enzyme and the phenolic compounds through an hidrogen link [60]. Moreover, the antifungic pure lignan effect of NDGA and methyl-NDGA has been tested against *A. flavus* and *A. parasiticus*, and obtainment form 82 to 100% of inhibition of the growth colony diameter, with a concentration of 300 to 500 µg/ml, observing a higher fungicide effect and during much time polger with the use of methyl-NDGA [64]. Vogt et al. (2013) [65], have studied the fungicide effect of NDGA against 4 fungi phyto-pathogens *F. graminearum* (MIC 62.5 µg/ml), *F. solani* (MIC 250 µg/ml), *F. verticillioides* (MIC 125 µg/ml) and *Macrophomina phaseolina* (MIC 125 µg/ml), and obtained a strong inhibitor effect in all cases. Tequida et al. (2002) [25], showed the inhibitor effect of the alcoholic extracts of *L. tridentata* against *A. flavus*, *A. niger*, *Penicillium chrysogenum*, *P. expansum*, *Fusarium poae* and *F. moniliforme* with a inhibition percentage within the range of 41.5 % to 100%. Also, the potential fungicide effect has been studied with the resin extract of creosote bush against *Rhyzoctonia solani* and *Fusarium oxysporum* [21].

1.6.4 Anti-viral properties

It is well known that the NDGA acts in a mechanical way as a lipoxygenase inhibitor interfering with the enzyme action [66]. It has been demonstrated that NDGA has an important effect in the cyclooxygenase pathway in the esophagus adenocarcinoma [67], while the methyl-NDGA is a potent agent inhibitor of the HIV virus replication by affecting the HIV transcription and simple herpes [68,69].

1.6.5 Insecticide properties

The potential use of some lignans as an insecticide has been reported early to obtain results compared with commercial synthetic pyrethroid activity, for an instance the sesquilignan Haedoxan A, isolated from the root of *Phryma leptostachya,* it is maybe the lignan well better known with insecticide properties, in combination with piperonyl butoxide, in synergy with pesticidal inhibitors of cytochrome P^{450}, it has an excellent insecticide activity for lepidoptera larvae and *Musca domestica*, DL_{50}=0.25 ng/fly. The physiological effects of these lignans are muscular relaxation, food cessation, general paralysis, and death, causing similar effects like nereistoxin [70]. Moreover, there are some lignans than have been used as an effective enhancing toxicity of a wide range of insecticides, attributable to the presence of the metilendioxi group, which is responsible of the enzymatic system that carry out the oxidation and inactivation of the majority of the toxins (cytochrome P^{450} oxidase) [42].

1.7 TOXICITY

Up to now, the majority of lignans seem not to be linked to intoxication problems in humans and animals, except lignans derivative of *Podophyllum* genus and NDGA. Although NDGA was used as an antioxidant for fat and butter in food in the USA for many years, it was retired by the FDA in 1968 for causing cystic nephropathy in rats. High doses of the plant extract showed hepatotoxicity, dermatitis and bile toxicity, this toxicity is eliminated when NDGA is O-methylated [71]. However, several results in studies in humans and rats support the non-toxic nature of these kinds of compounds at 300 mg/kg [69,72].

1.8 SYNERGY BETWEEN CHEMICAL ELEMENTS OF THE PLANT

Besides the NDGA, the other half of the resin has more than 20 flavonoids and methyl aglycones. The possible effects of all different flavonoids and other constituents are numerous and varied. The combined effects of these constituents point out to a synergism that amplifies the composed primary effect (NDGA), this suggests the advantage of using an extract of the whole structure leaf/branches in comparison to using a NDGA purified and synthesized preparation [27].

1.9 USE OF *Larrea tridentata* IN FARM ANIMAL PRODUCTION

As mentioned before in detail, creosote bush (*Larrea tridentata*) shows many and diverse uses according to the well documented research. Information about the use of creosote bush in farm animal production is scarce, in fact it could be said that its null. The first study with creosote bush was done by Duisberg (1952) [6] he found that both the bitter taste and the disagreeable odor of creosote bush leaves could be removed by extraction with ethyl alcohol. The residual meal after drying was edible for sheep, cattle, and goats without any additions. The carcass of a wether, fed extracted creosote bush meal for 30 days, was found to be fat in good condition, and did not possess any unusual odors or flavors. Milk from a cow and a goat fed the meal for ten days was of good quality and of normal flavor [6]. In a study by Martínez (2016) [73] the effect of aqueous extract of *L. tridentata* was evaluated on the quality of poultry litter as an ingredient for beef

cattle. Results showed a decrease on coliforms, molds and yeast count in the poultry litter treated with creosote bush extract also there was that a higher *in vitro* digestibility of neutral detergent fiber, so the use of the extract could be used to reduce the load of poultry litter microorganisms used to feed beef cattle without affecting feed digestibility. In another experiment by García et al. (2018) [74] the aim of the study was to evaluate the effects of dietary addition of whole plant, leaves, and a powder aqueous extract of *Larrea tridentata* on growth, organ weights and serum hepatic enzymes of broilers. Broilers fed the whole plant, leaves or powder aqueous extract had higher body weight and daily weight gain than control. Powder aqueous extract of *L. tridentata* reduced crop, gizzard and liver weights as compared to control, whole plant, and leaves. Broilers fed powder aqueous extract had the lowest serum hepatic enzyme concentration as compared to the control and those fed the whole plant and leaves.

In another experiment by Faz et al. (2017) [75] the aim of the study was to evaluate the effect of *Larrea tridentata* whole plants on some traits of fatten lambs. 21 weaned Rambouillet lambs were used. Control treatment had higher final weight than treatments with *L. tridentate* inclusion. However, there were no differences for daily weight gain for all treatments. Ruminal pH values were similar for all treatments. On carcass traits, cold carcass weight, warms carcass weight and carcass longitude were higher for the control treatment. The loin perimeter was higher for the 10% inclusion *L tridentata* treatment. Moreover, Hernández et al. (2019) [76] determined the effect of integrated diets with creosote bush biomass added as fodder for sheep intake, weight gain, pH, rumen protozoa and concentration of *Eimeria spp*, oocysts in feces. The sheep fed 5 to 10 % creosote bush had lower feed intake than the control. Weight gain, feed efficiency and rumen pH had values with non-significant differences for all treatments. The number of rumen protozoa and oocysts in feces decreased as the percentage of creosote bush in the diet increased. López et al. (2020) [35] performed a study where extracts of creosote bush leaves were evaluated for control of *Salmonella typhimurium* in poultry pens. Solvent and the technique that obtained the highest proportion of extractable material, in vitro, were determined; its bactericidal capacity was determined and the capacity to disinfect poultry pens was evaluated, comparing disinfection with creosote bush, routine disinfection, and sanitary vacuum. At the end of the disinfection, samples were collected in floor, wall, ceiling, and curtain. Seeded in selective medium, differential biochemical tests were performed and colony forming units (CFU) were calculated. On the wall, the CFU with creosote bush decreased, while in ceiling and curtains they were similar with the routine disinfection and on the floor, lower with routine disinfection. In another study by Mejía (2020) [77] the objective was to evaluate the effect of different concentrations of *Larrea tridentata* on broilers performance and three hepatic enzymes (ALT, AST, ALP) and three proteins (albumin, globulin and total protein). The results did not show a significant difference in terms of productive variables, and hepatic enzymes in the different doses compared with the control. A decreasing linear trend was shown in albumin and total protein values as the dose of the extract increased.

1.10 CONCLUSION

It can be observed that the use of creosote bush (*Larrea tridentata*) has been varied, and this perennial bush has been studied for more than seven decades. Of all uses given to this plant, the less studied is as a part of the animal farm production, the creosote bush metabolites such as NDGA have a strong potential in animal husbandry. Nevertheless, the few studies performed with sheep and broilers, they open an opportunity to use this plant in this area. More studies are warranted in these and other animal species to corroborate the promising results found till this moment.

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2. DIAGNOSTICO DE LA PRESENCIA DE *Varroa destructor* EN SAN LUIS POTOSÍ Y FACTORES AMBIENTALES QUE PUEDEN FAVORECER SU DESARROLLO

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2.1 RESUMEN

Los factores ambientales, al igual que las practicas de manejo del apicultor son un importante factor de influencia en el desarrollo de las poblaciones de *Varroa destructor* al igual que su interacción con *Apis mellifera*. Con el objetivo de determinar el porcentaje de infestación y prevalencia de *V. destructor* en colonias de *A. mellifera* en la región altiplano, centro y media del estado de San Luis Potosí, así como la relación de dicha parasitosis con variables geográficas y ambientales, se realizo el muestreo de 284 colmenas en 71 apiarios ubicados en las regiones mencionadas, encontrando que la presencia de varroa para las colmenas muestreadas fue del 100%. El porcentaje de infestación promedio para las tres regiones del estado fue de 3.8%, sin encontrar diferencias significativas entre regiones, municipios, ni zonas climáticas. No se encontró correlación significativa entre las variables ambientales ni la altitud con el porcentaje de infestación de varroa. Sobre los productos utilizados para el control del varroa, el químico sintético amitraz en el más utilizado por los apicultores (59%), seguido por el aceite esencial de orégano (17%) y acido oxálico (11%). Finalmente, los apicultores que muestran el menor porcentaje de infestación en sus apiarios (3%) son aquellos que refieren el uso de amitraz, seguidos de los que aplican acido oxálico con un promedio de infestación de varroa de 3.9%.

2.2 INTRODUCCIÓN

América Latina es una de las regiones con mayor producción y exportación de miel en el mundo; destacando países como Argentina y México, lo cuales tienen una fuerte tradición apícola. México se ha consolidado dentro de los primeros 10 productores y ocupa la tercera posición como

exportador de miel, por lo que se considera a la apicultura como una de las principales actividades pecuarias generadora de divisas para el país que beneficia al sector rural, especialmente en zonas marginadas, donde la ganadería no se desarrolla de forma extensiva (Medina-Flores *et al*., 2014). Durante el 2020, la producción nacional alcanzo un total de 54,122 ton, de los cuales un 48% se destinó a la exportación principalmente a países como Alemania, Estados Unidos, Reino Unido y Arabia Saudita (SIAP, 2020). En el país cerca de 45 mil productores se dedican a la apicultura, atendiendo 1.9 millones de colmenas. San Luis Potosí al igual que el resto del país experimenta una disminución en la eficiencia productiva asociada a factores de riesgo como el retraso y menor duración de las épocas de floración; y la presencia de enfermedades parasitarias principalmente *Varroa destructor*, cuya frecuencia a nivel nacional supera el 80% (UNAM y SENASICA, 2017). *V. destructor* es considerado el parasito más destructivo para la abeja de la miel, ya que se alimenta de la abeja en etapas inmaduras y adultas, provocando importantes alteraciones en su comportamiento, malformaciones, disminución de su esperanza de vida (Elis, 2018), supresión de la respuesta inmune (Beaurepaire *et al.,* 2020) e incluso llega a conducir al colapso de la colonia (Meikle y Diaz, 2012), pues ha sido detectada en el 98% de las colonias afectadas por el síndrome de colapso de la colmena (Locke *et al*., 2012), además de considerarse un fuerte predictor de la reducción invernal de la población de abejas en Europa y USA (Dainat *et al*., 2012). Se cree que dicho impacto catastrófico se atribuye a su función como vector biológico de diferentes virus de ARN (Beaurepaire *et al*., 2020; Mondet *et al*., 2014; Yang y Cox 2005) que rápidamente resulta en el desarrollo de epidemias virales letales que regularmente conducen a la muerte de la colonia en un lapso de dos a tres años (Amdam *et al*., 2004).

Se sabe que la relación entre la abeja de la miel y varroa está fuertemente influenciada por factores ambientales. Uno de ellos corresponde a las practicas del apicultor, tales como como la selección de abejas, el uso de acaricidas, la actividad migratoria, la comercialización de abejas, el intercambio de bastidores entre colonias, entre otras (Dynes *et al.,* 2020; Neumann y Blacquiére, 2017), ya que estas favorecen la transmisión horizontal del ácaro y al igual que la selección de variantes más virulentas (Fries y Camazine, 2001). Por su parte, las variaciones en temperatura y humedad relativa tienen un impacto directo sobre la tasa de crecimiento de la población de varroa, al afectar sus condiciones para la reproducción y su mortalidad, y probablemente las diferentes tasas de desecación (Bruce *et al.*, 1997). Le Conte y Desenfant (1990) señalan que la reproducción óptima de varroa se da a un rango de temperatura de 32.5-33.4°C, viéndose reducida a temperaturas superiores a los 36°C; y favorecida a un porcentaje de humedad relativa de 70% en comparación con un 40%, y muriendo cuando la temperatura excede los 38°C. Por otra parte, datos experimentales han demostrado que la supervivencia del ácaro disminuye a temperaturas por debajo de 33°C, mientras que un rango de 34°a 35°C incrementa su supervivencia y favorece su reproducción (Gao, 2002). Esto concuerda con los observado por van Engelsdorp *et al*. (2008), quienes describen que las regiones con temperaturas relativamente bajas presentan un menor porcentaje de perdida de colmenas. De igual forma, el impacto de las variaciones climáticas puede extenderse más allá de los efectos directos; por ejemplo, llegando a interrumpir el proceso de maduración de las hembras de varroa y reducir su fertilidad, por consecuencia se incrementa la mortalidad de descendencia inmadura (Ifantidis *et al.,* 1999) que a lo largo del tiempo disminuirá el porcentaje de ácaros fértiles (Martin, 2001). Esta disminución de la distribución de hembras adultas de varroa dentro de la colonia por largos periodos de

tiempo reducirá también el número promedio de ciclos reproductivos intentados por la hembra durante su vida (Harris *et al.* 2003). Por lo tanto, una colonia de *A. mellifera* sobreviviente a varroa, al ser evaluada dentro y fuera de su lugar de origen, puede presentar un nivel de infestación diferente debido a la fuerte influencia de los factores ambientales, dejando clara la presencia de interacciones genotipo-ambiente (Meixner *et al.*, 2015).

Por lo tanto, el objetivo del presente estudio es determinar el porcentaje de infestación y prevalencia de *V. destructor* en colonias de *A. mellifera* en la región altiplano, centro y media del estado de San Luis Potosí, así como la relación de dicha parasitosis con variables geográficas y ambientales.

2.3 MATERIALES Y METODOS

2.3.1 Ubicación y muestreo

El estado de San Luis Potosí cuenta con un padrón de 333 apicultores (SINIIGA, 2019), en las regiones Altiplano, Centro y Media del estado, mismas que se cree presentan un manejo similar en sus unidades apícolas. Esta región cuenta con una prevalencia esperada para varroa de 84% (UNAM y SENASICA, 2017). Con base en esta información se estableció un muestreo estratificado. Durante los meses de agosto a noviembre de 2020 muestras de 71 apiarios fueron colectadas, considerando el 15% de las colonias del apiario, para un total de 284 colmenas muestreadas. Se registraron las coordenadas geográficas para cada sitio.

La muestra se distribuyó de la siguiente manera; 27 apicultores para la zona altiplano, 24 zona centro y 20 zona media, seleccionados de manera aleatoria en 13 municipios del estado entre los que se comprende Villa Hidalgo, Matehuala y Venado de la región altiplano; Ahualulco, Mexquitic de Carmona, Soledad de Graciano Sánchez, Tierra Nueva, Villa de Arriaga, Villa de Reyes y Villa de Zaragoza de la parte centro; y Cerritos, Rioverde y San Ciro de Acosta de la zona Media. Dichos municipios se ubican dentro de las seis zonas climáticas (figura 1) que se describen a continuación:

- Muy seco templado con lluvias en verano BWkw con una temperatura media anual entre 16 y 20°C, y una precipitación total anual de 100 a 400 mm.
- Seco semicálido con lluvias en verano BS_0KW que registra una temperatura media anual de 18.7 a 21.8°C y una precipitación total anual de 28 a 422 mm.
- Seco templado con lluvias en verano BS_0kx' con una temperatura media anual promedio de 16 a 18°C y una precipitación total anual de 300 a 400 mm.
- Semiseco semicálido BS_1hw que se presenta en la parte central del estado, con una temperatura media anual entre 18.8 y 21.7°C, con una precipitación total anual de 495 a 645 mm.
- Semiseco templado BS_1kw, este clima se manifiesta en la porción sur del estado. La temperatura media anual promedio está dentro de los rangos de 16.1 a 17.6°C y la precipitación total anual va de los 350.5 a 577.7 mm.

- Templado subhúmedo con lluvias en verano C(wo)(x') ubicado en el norte del estado, registra una temperatura media anual de entre 12 a 16°C, y la precipitación total anual es de 500 a 700 mm (INEGI, 2007).

Las colonias que formaron parte del muestreo presentaban reinas de diferente edad y origen, y tanto el manejo sanitario como productivo era diferente por pertenecer a diferentes productores.

2.3.2 Estimación del porcentaje de infestación de varroa

La estimación del grado de infestación con *V. destructor* en abejas adultas se realizó utilizando el método del frasco descrito por De Jong y De Jong (1983) que consiste en colectar de los bastidores de la cámara de cría de la colmena una muestra aproximada de 250 abejas adultas que son depositadas y agitadas en un frasco con alcohol al 75% para desprender los ácaros adheridos al cuerpo de la abeja. Posteriormente, el contenido del frasco es vertido en una coladera y se contabiliza la cantidad de ácaros y abejas. El porcentaje de infestación se determinó al dividir en número de ácaros obtenidos entre el número de abejas por muestra y después se multiplicó el resultado por 100.

2.3.3 Monitoreo de variables ambientales

La temperatura ambiental mínima y máxima, al igual que el porcentaje de días con temperatura ≥ a 35°C fueron obtenidos de la estación meteorológica de la Comisión Nacional del Agua más cercana, considerando los 15 días anteriores a la fecha de muestreo. Sobre las prácticas de manejo únicamente se registró el producto que utiliza el apicultor para el control de *V. destructor*.

2.3.4 Análisis estadístico

En el análisis estadístico, la variable de respuesta fue el porcentaje de infestación con *V. destructor*, considerando las regiones, municipios, zonas climáticas y productos aplicados como tratamientos bajo un diseño completamente al azar. Para la comparación del nivel de promedio de infestación entre regiones, municipios, zonas climáticas y productos aplicados para control de varroa, se utilizó un análisis de varianza y posteriormente una prueba de comparación de medias de Tukey ($P<0.05$) cuando fue necesario. Para evaluar la asociación entre las variables climáticas (temperatura mínima, máxima, porcentaje de días con temperatura ≥ a 35°C y altitud) y el nivel de infestación de varroa, se aplicó un análisis de correlación de Kendall para variables no paramétricas.

Para cumplir la suposición de normalidad, los datos sobre el porcentaje de infestación de varroa fueron convertidos a LogN y los resultados fueron presentados como medias expresadas en las unidades originales.

2.4 RESULTADOS

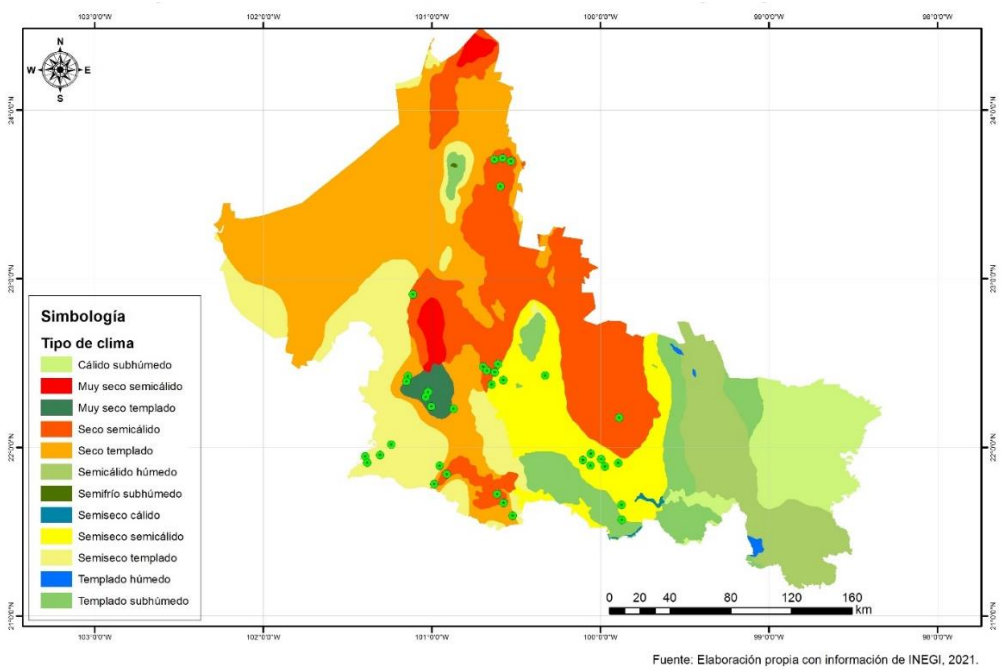

Figura 2.1 Distribución de sitios de muestreo de colonias de *A. mellifera* en las diferentes regiones climáticas del estado.

La presencia de *V. destructor* fue del 100%, encontrándose en todas las colmenas muestreadas. El porcentaje de infestación promedio para las tres regiones del estado fue de 3.8%. No se encontraron diferencias significativas entre las tres regiones económicas del estado (altiplano, centro y media) ni entre los 14 municipios muestreados como se muestra en la tabla 1.

Tabla 2.1 Porcentaje de infestación de *V. destructor* en tres regiones económicas del estado y 14 municipios de San Luis Potosí.

Región	% Infestación	Municipio	% Infestación
Altiplano	3.4 a	Villa Hidalgo	3.3 a
		Matehuala	3.6 a
		Venado	4.0 a
Centro	3.6 a	Ahualulco	1.2 a
		Mexquitic de Carmona	2.6 a

		Soledad de Graciano Sánchez	3.8 $_a$
		Tierra Nueva	6.8 $_a$
		Villa de Arriaga	3.5 $_a$
		Villa de Reyes	4.8 $_a$
		Villa de Zaragoza	1.3 $_a$
		Cerritos	1.9 $_a$
Media	4.8 $_a$	Rioverde	5.1 $_a$
		San Ciro de Acosta	4.9 $_a$

[a,b] Diferentes literales en una misma columna indican diferencias significativas a un nivel de $P<0.05$.

Sobre sobre el nivel de infestación de varroa en las 6 diferentes zonas climáticas comprendidas en el muestreo no se encontraron diferencias significativas (tabla 2). Sobre la aplicación de productos para el control de varroa, el producto más utilizado es el amitraz (Apivar®) siendo aplicado en un 59% de las colmenas muestreadas, en segundo lugar, se encuentra la aplicación de aceite esencial de orégano (17%), posteriormente tenemos el uso de ácido oxálico (11%), timol (6%), flumetrina (Bayvarol®, 4%) y solo un 3% no aplica ningún tipo de tratamiento.

Se encontraron diferencias significativas en el porcentaje de infestación de varroa según el tipo de producto utilizado por el apicultor, siendo las colmenas tratadas con amitraz y ácido oxálico las que presentan los porcentajes más bajos de infestación por varroa (3.0% y 3.9% respectivamente), mientras que los apicultores que utilizan aceite esencial de orégano (*Lippia berlandieri*), timol y flumetrina mantienen porcentajes de infestación de varroa similares a las colmenas que no reciben ningún tratamiento, tal como se muestra en la tabla 2.

Tabla 2.2 Análisis de varianza sobre el porcentaje de infestación de varroa en diferentes zonas climáticas y productos acaricidas en San Luis Potosí.

Zona climática	% Infestación	Producto acaricida	% Infestación
Muy seco templado	2.3 $_a$	Amitraz	3.03 $_a$
Seco semicálido	3.9 $_a$	Acido Oxálico	3.90 $_a$
Seco templado	3.6 $_a$	Timol	4.07 $_b$
Semiseco semicálido	4.2 $_a$	Aceite esencial oregano	4.76 $_b$

Semiseco templado	3.9 $_a$	Flumetrina	7.31 $_b$
Templado subhúmedo	6.0 $_a$	Sin tratamiento	10.18 $_b$

[a,b] Diferentes literales en una misma columna indican diferencias significativas a un nivel de $P<0.05$.

Referente a la correlación entre variables ambientales y el porcentaje de infestación de varroa, para la variable altitud no presentó correlación significativa.

2.5 DISCUSIÓN

Respecto al nivel de infestación promedio en las regiones estudiadas del estado, este se encuentra dentro de los valores considerados como umbral económico (UE) por diferentes autores y a partir del cual se recomienda la aplicación de tratamiento para el control de varroa. Por ejemplo, Delaplane y Hood (1999) en una época similar del año en Georgia y Carolina del Sur, consideraron que el UE se encuentra entre un 5 a 12.67% de infestación. Por su parte, Strange y Sheppard (2001) establecen un umbral de 3% durante la primavera, 14% en verano y 3% en otoño. De manera similar Currie y Gatien (2006) recomiendan la aplicación de tratamientos para el control de varroa a partir de 2% en primavera y 4% en verano, estableciendo estos valores como umbral económico en la parte sur de Canadá. Algunos grupos involucrados en el sector apícola, como la Honey Bee Health Coalition (2018) menciona que dicho umbral se encuentra alrededor del 3% de infestación, aun cuando de acuerdo con Dietemann *et al.* (2012) existe una gran falta de investigación en este sentido.

Por otra parte, al comparar nuestros resultados con estudios realizados en condiciones climáticas similares en el estado de Jalisco, podemos observar que se encuentran por debajo del nivel de infestación promedio (5.2%) obtenido para zonas templadas y cálidas (Tapia-González *et al*. 2019). Y también es menor al 5.02% señalado por Medina-Flores *et al.* (2014) para el estado de Zacatecas en la temporada de otoño en tres zonas climáticas (semiseca semicálida, semiseca templada y subhúmeda templada). Por el contrario, nuestros resultados superan los niveles de infestación obtenidos por Ruíz-Flores *et al.* (2012) al evaluar la zona rural del Distrito Federal y Estado de México en clima templado subhúmedo principalmente, al menos durante los primeros cuatro años de su estudio, donde encontraron valores de 3.08% en 2002, 2.31% en 2003, 3.61% en 2004, 2.82% en 2005, y finalmente 4.51% en 2006, estimando una tasa media de incremento anual de 0.34 ± 0.23 %.

Szabo y Walker (1996) mencionan que el desarrollo poblacional de *V. destructor* puede verse favorecido por la mayor continuidad que existe en la disponibilidad de cría de *A. mellifera* en zonas de clima cálido y húmedo en comparación con climas templados. Sin embargo, el nivel de infestación promedio de 3.8% obtenido en el presente estudio en las seis zonas climáticas evaluadas, es superior al reportado por Martínez-Puc *et al.* (2011) para la localidad Xmatkuil en el municipio de Mérida, Yucatán en condiciones de clima cálido subhúmedo con un promedio de

1.7% en colonias manejadas y 1.96% en colonias silvestres, por lo que las condiciones ambientales parecen no ser el factor decisivo en el nivel de infestación de varroa. En este sentido Medina-Flores *et al.* (2014) al analizar el efecto del genotipo y la zona ecológica sobre la infestación con varroa, observaron en primer lugar, que la prevalencia al igual que el nivel de infestación de varroa fue significativamente mayor en colonias de genotipo europeo en comparación con aquellas de genotipo Africano, independientemente de la región climática. En segundo lugar, encontraron un mayor grado de africanización en las colonias de regiones subtropicales y templadas sub-humedas, y una mayor tendencia hacia el genotipo europeo en regiones templadas y secas, revelando que existe un efecto ambiental sobre la introgresión del gen africano en las colonias de abejas, principalmente asociado a la mayor capacidad de adaptación de las abejas africanizadas a climas tropicales y subtropicales. Finalmente, concluyeron que el efecto del genotipo representa el factor de mayor influencia en el nivel de infestación a pesar de la región climática, especialmente con los genotipos africanos debido a su menor susceptibilidad frente a varroa como lo mencionan Moretto y Leonidas (2003). Dicha hipótesis concuerda con lo observado por Tapia-González *et al.* (2019) y Medina-Flores *et al.* (2014) al igual que en el presente trabajo, donde en ninguno de los casos se encontraron diferencias estadísticas entre los niveles de infestación de las diferentes zonas climáticas, además de considerar que al ser un estudio a escala regional es posible que no exista una variación significativa entre el genotipo de las colonias de abejas que se manejan en esta región.

Respecto al porcentaje de infestación de varroa en relación con el acaricida aplicado, los resultados sugieren que los apicultores que utilizan amitraz y ácido oxálico presentan los porcentajes de infestación más bajos y son estadísticamente similares, tal como lo mencionan Reyes *et al.* (2020) quienes observaron una eficacia similar entre ambos productos; sin embargo al contrastarlos con el timol, este presento una mejor respuesta para el control de varroa. Anteriormente, elevados porcentajes de efectividad del ácido oxálico han sido documentados; Díaz-Monroy *et al.* (2019) compararon diferentes productos acaricidas, obteniendo una mayor eficacia (84.4%) para el ácido oxálico en comparación con el timol (62.9%). Respecto a la efectividad del aceite esencial, Romo-Chacón *et al.* (2016) evaluaron la efectividad del aceite esencial de orégano como una alternativa orgánica al amitraz, encontrando una mejor respuesta para el amitraz con una eficacia de 97% y de 57 a 74% para el aceite esencial. En este sentido, es importante mencionar que los efectos de los aceites esenciales sobre la conducta de varroa han sido definidos como de atracción, repelencia o toxicidad, por lo que es posible que solo lleguen a generar un efecto desorientador sobre la varroa (Kraus *et al.*, 1994).

Además de la efectividad del producto en sí, se debe considerar que existen otros factores que influyen en el resultado de un acaricida, tales como la época de aplicación, el nivel de concentración de los ingredientes, frecuencia de aplicación, selectividad, condiciones climáticas, condiciones biológicas de la colmena, calidad genética, calidad de manejo, historial de aplicaciones químicas o incluso el desarrollo de mecanismos de resistencia debido al uso inadecuado del producto durante periodos de exposición continuos y prolongados (Martínez-Puc y Medina-Medina, 2011), tal como lo mencionan Rodríguez-Dehaibes *et al.* (2005) quienes evaluaron la resistencia de poblaciones de varroa en el estado de Veracruz frente a productos como Amitraz y flumetrina; al analizar los resultados concluyeron que la DL_{50} en varroa para la

flumetrina era 327 veces mayor a la establecida nueve años atrás en México, mientras que la DL_{50} para Amitraz fue tan solo 2.3 veces mayor que la determinada anteriormente. Por lo que es posible que la eficacia de productos a base de flumetrina haya disminuido debido a la generación de resistencia, influyendo para ello el que sea uno de los primeros productos químicos disponibles en México para el control de varroa.

2.6 CONCLUSIONES

Los programas de manejo y control de varroa empleados por los apicultores de la zona altiplano, centro y media del estado de San Luis Potosí parecen ser efectivos al momento de mantener los porcentajes de infestación dentro de su umbral económico, sin embargo, es importante considerar que aproximadamente una cuarta parte de las colonias muestreadas rebasa dicho umbral, siendo probable que ya presenten pérdidas económicas generadas por esta parasitosis. En este sentido, es evidente el interés de los apicultores por descartar el uso de acaricidas químicos sintéticos y optar por opciones naturales que no representen ningún riesgo para la colonia de *A. mellifera*, ya que un 34% hace uso de este tipo de productos, aun cuando la variación en su calidad compromete su eficacia y dificulta el alcance de resultados óptimos en el control de varroa, por lo que el producto químico amitraz continua siendo el más popular entre los apicultores, además de presentar los porcentajes más bajos de infestación al igual que el ácido oxálico. Por otra parte, ninguno de los factores climáticos evaluados parece estar relacionado con el nivel de infestación de varroa de las colonias en estudio, al igual que la zona climática en que se ubican los apiarios; sugiriendo que existen otros factores que influyen en mayor medida en el desarrollo de las poblaciones de varroa dentro de la colmena.

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3. *Varroa destructor*: ITS INTERACTION WITH *Apis mellifera* COLONY STRENGTH IN TWO LOCALITIES OF SAN LUIS POTOSÍ, MEXICO

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3.1 ABSTRACT

The mite *Varroa destructor* is one of the greatest threats to the apiculture sector worldwide. Generation of knowledge on its behavior and of the factors that favor its development under different environmental conditions, as well as the level of influence of these factors, is essential to the development of strategies for integral management of the mite. With the objective of analyzing the interaction between the variables of *Apis mellifera* colony strength and the percentage of *V. destructor* infestation, a group of 40 beehives were monitored during two flowering seasons, spring, and summer-fall, in San Luis Potosí, Mexico. The variables were analyzed using the Pearson correlation analysis as well as an analysis of variance with repeated measures in time ($p \leq 0.05$). The level of *V. destructor* infestation was significantly related to the quantity of honey (+0.58) and pollen (+0.62) reserves and negatively correlated with hygienic behavior (-0.65). The percentage of *V. destructor* infestation in spring was not significantly different from that in summer-fall, while the quantity of food (honey and pollen) reserves in spring were statistically superior as of the second half of the season. Hygienic behavior had statistical differences only at the beginning of the flowering seasons; it was superior in summer-fall. However, this difference was not maintained over time.

Key words: level of infestation, hygienic behavior, flowering season.

3.2 INTRODUCTION

In Mexico, apiculture is one of the animal production activities that generates the most foreign currency for the country, reaching 65 million dollars in 2020 (SIAP, 2020). This activity benefits the rural sector, especially in marginalized areas where livestock production is not extensive (Medina-Flores *et al.,* 2014). San Luis Potosí is in a privileged position in Mexico where three different apicultural regions converge. This endows the region with great potential to produce

honeys from different flowers. However, like the rest of the country and other regions of the world, bees face numerous challenges, among them is the presence of the ectoparasite *V. destructor*, considered by many researchers to be one of the most significant sanitary problems for *Apis mellifera* colonies worldwide (McMenamin & Genersch, 2015). At the national level, its frequency is more than 80%, and an annual loss of more than 33% of the bee colonies on the central high plateau and in the northern regions of the country is associated with the presence of the mite (Medina-Flores *et al.,* 2018). Its impact on management and profitability of apiculture is due to the major alterations that it causes in bee behavior, malformations, reduction in life expectancy and in honey production. However, the most catastrophic effect of the mite is its function as a biological vector of different RNA viruses and as a suppressor of bee immune response (Beaurepaire *et al.,* 2020).

Growth of the population of *V. destructor* depends on the balance between the rate of reproduction and the rate of mortality of the individuals inside the beehive at a given moment. However, they are affected by *A. mellifera*'s different mechanisms of resistance and tolerance, such as reduction in population growth, reduction of the post-capping period, and low fertility, fecundity and reproductive success of the founding females, grooming, hygienic behavior, cell size, self-medication, social apoptosis, attractiveness, and low susceptibility of the swarm play an important role in regulating the mite population (Strauss *et al.*, 2015).

Hygienic behavior is the ability of the workers to detect sick or parasitized larvae, discriminate between normal and abnormal larvae, and remove the cap and the sick larvae or the parasite. Even when this removal does not guarantee death of the mite, it can interrupt its reproductive cycle and decrease female fertility (Mondet *et al*., 2020). Also, environmental conditions such as variations in temperature and relative humidity, beekeeper management practices, availability of pollen and flow of nectar, long brooding periods, and number of drone larvae can induce a drastic increase in the size of the mite population (Le Conte & Navajas, 2008).

To design strategies that tend to control and reduce the occurrence of this parasitosis in *A. mellifera* populations, it is necessary to quantify it, identify the factors associated with colony strength, apiary management and the different environments in which apiculture is practiced that contribute to the presence and development of *V. destructor*. The strength of a colony is understood to be the result of the interaction between the demography of the colony, energy sources, and the temporal and spatial pattern of availability of environmental resources in the landscape (EFSA-AHAW Panel, 2016). Therefore, the objective of this study was to analyze the role of the different factors associated with the strength of a colony that affect the *A. mellifera – V. destructor* interaction during two flowering seasons in two localities of San Luis Potosí, México.

3.3 MATERIALS AND METHODS

3.3.1 Monitoring scheme

In 2020, a group of 40 *A. mellifera* beehives in the High Plateau and Central regions of the state of San Luis Potosí were monitored during two flowering seasons. Spring (April-May) monitoring was carried out in the locality of El Mezquite, municipality of Villa de Arista at the coordinates

22° 40' 46.10" N and 100° 55' 4.85" W, altitude 1621 m. Climate is dry and warm with an average temperature of 22.8 °C, low of 13.5 °C and high of 32.1 °C. Total precipitation during the sampling period was 13.8 mm. Here, the mesquite (*Prosopis laevigata*) is the species of greatest importance for apiculture of the season. Later, at the beginning of the summer-fall season (August-October) the same group of beehives were transported to the locality of El Mezquital, municipality of Villa de Arriaga, at the coordinates 22 °7' 45.61" N and 101° 16' 33.62" W, altitude 2160 m; climate is semiarid temperate with mean temperature of 16.5 °C, low 8.7 °C and high 24.4 °C. Total precipitation during the period was 76.4 mm. This season is characterized by a broader diversity of floral resources, although *Bidens odorata* is the species recognized traditionally as the most important of the season. The data on low and high temperatures during the sampling periods were requested from the Coordinación General del Servicio Meteorológico Nacional, CONAGUA, for the weather stations closest to the location of the apiaries (24098 and 32127).

The sampling period was 45 days; according to the knowledge and experience of local migratory beekeepers, this is the average resident time of a beehive in a site during one harvest. Sampling was carried out every two weeks during each flowering season. The monitored beehives had a Jumbo-type brood chamber (46.5 cm long, 38 cm wide and 29.5 cm high) with capacity for 10 frames and no honey super. For each beehive, we used one commercial freely fertilized Italian queen in her first year of production. Before monitoring, the queens were introduced to orphan beehives in wooden cages for their recognition. On the third day after introduction, the candy stopper (mixture of powdered sugar and honey) that obstructed the exit from the cage was perforated with a wooden toothpick to stimulate the worker bees to eat the candy and conclude the release of the queen. Finally, one week later, a routine inspection of the hive was carried out to verify that the queen had initiated oviposition. The hives had homogeneous conditions in terms of honey and pollen reserves, brood, and adult bee population, as well as statistically similar levels of *V. destructor* infestation. The parameters in the brood chamber of all the hives were estimated using the method described below.

Brood solidness (BS): This pattern was determined by placing a grid delimiting 100 cells over a section of capped brood. Empty cells were then subtracted to estimate the percentage of brood solidity. The procedure was repeated on different patches of brood to derive a mean of at least ten observations.

Adult bee population (ABP): The adult bee population was determined by the difference in weight in kilograms between the complete hive during the night and the sum of the weight of its elements without adult bees.

Hygienic behavior (HB): The percentage was estimated using the technique of perforating 50 capped brood cells using an entomological pin #00. The area was delimited with colored pins, and 24 h later it was inspected again to estimate the percentage of removed brood.

V. destructor infestation percentage (VDIP) in adult bees: This was determined by the jar method described by De Jong *et al*. (1982).

Worker brood (WBR), drone brood (DBR), capped honey (CH), and pollen stored in the brood chamber (POL): Each of these was determined as the area in square centimeters occupied by each type of input by analyzing photographs of all the frames in the brood chamber (Figure 1) using the ImageJ tool of the National Institute of Health or by counting individual cells.

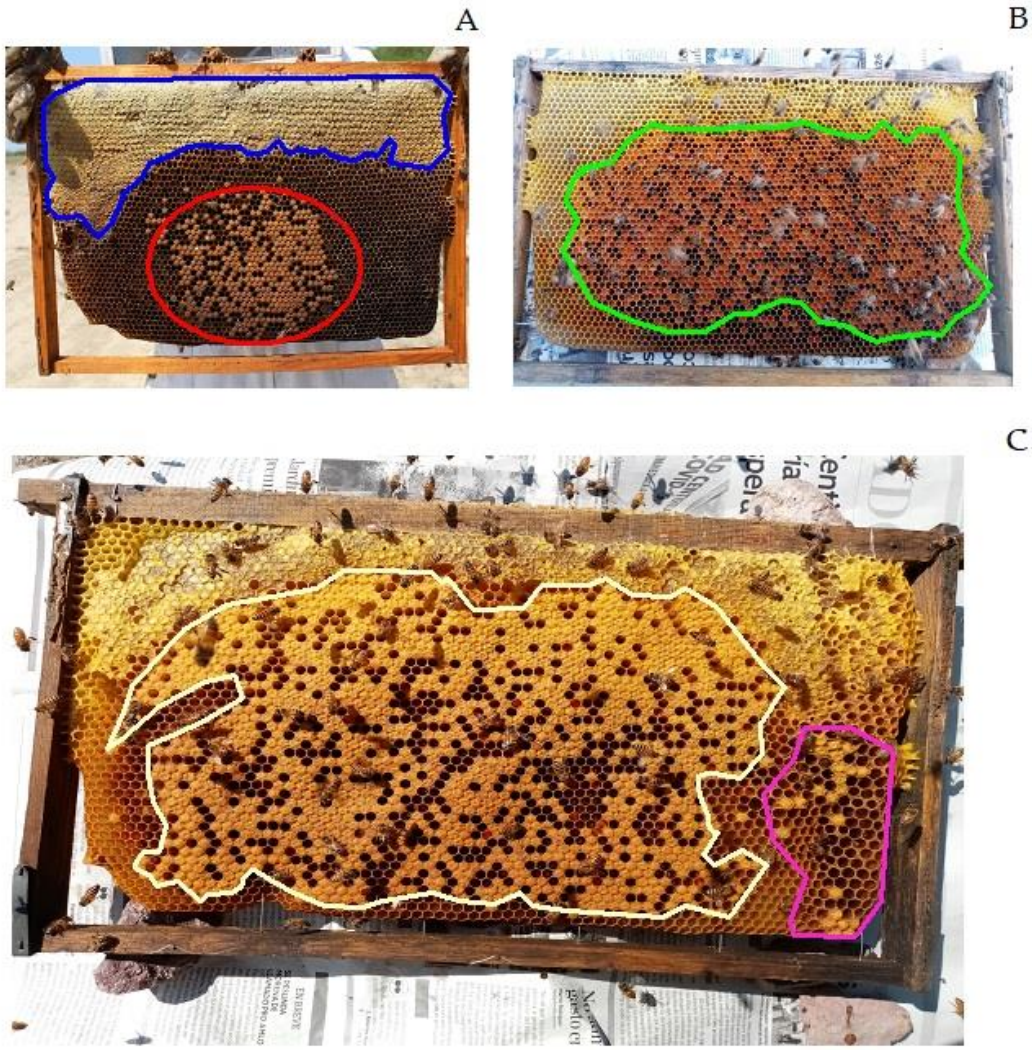

Figure 3.1 Variables evaluated in the brood chamber. A: capped honey outlined in blue; worker brood outlined in red; B: stored pollen marked in green; C: worker brood inside white line; cells of the drone brood outlined in pink are larger and projected outward.

3.3.2 Statistical analysis

Data were analyzed with the SAS tool OnDemand for Academics: Studio. Initially, a bivariate analysis was performed with Pearson correlation ($p \leq 0.05$) to establish the degree of association between the study variables. An analysis of variance with repeated measures ($p \leq 0.05$) was then applied considering two treatments: spring (Villa de Arista site) and summer-fall (Villa de Arriaga

site). For each treatment, the 40 hives were considered repetitions. This analysis was applied to only the variables that had significant correlations. To satisfy the assumption of normality, the data on capped honey and worker brood were transformed to square root, the quantity of pollen to logarithm, and the percentage of *V. destructor* infestation to arcsine of the square root. The results for these variables were presented as means expressed in transformed units.

3.4 RESULTS AND DISCUSSION

The results of this study enable analysis of the complex relationship between *A. mellifera* and *V. destructor*. The correlation analysis shows that only the variables honey (+0.58) and pollen (+0.62) reserves, as well as hygienic behavior (-0.65), correlated significantly with the level of mite infestation (Table 1).

Table 3.1 Pearson correlation between variables of colony strength and percentage of *V. destructor* infestation.

	CH†	POL	WBR	DBR	BS	HB	ABP
POL¶	0.65þþ						
WBR§	-0.15	0.24					
DBRþ	0.29	0.10	0.27				
BS¤	-0.22	-0.02	-0.02	-0.14			
HB††	-0.66þþ	-0.54	-0.34	-0.20	0.17		
ABP¶¶	-0.56þþ	-0.00	0.36	-0.20	0.40þþ	0.27	
VDIP§§	0.58þþ	0.62þþ	0.33	0.24	0.07	-0.65þþ	-0.16

†CH: capped honey; ¶POL: stored pollen; §WBR: worker brood; þDBR: drone brood; ¤BS: brood solidness; ††HB: hygienic behavior; ¶¶ABP: adult bee population; §§VDIP: *V. destructor* infestation percent. þþSignificant correlation at $p \leq 0.05$.

Regarding the level of *V. destructor* infestation, no significant differences were found between seasons during the 45 days of monitoring (Figure 2a), likely because the differences in climatic conditions of the two flowering seasons were not so accentuated or significant. As Szabo & Walker (1996) mention, the populational development of the mites may be more favored by the continuity that exists in the availability of *A. mellifera* broods in hot, humid climate regions and not in temperate climates, as is the case of this study. Medina-Flores *et al.* (2014) and Tapia-González *et al.* (2019) coincide with this hypothesis since they did not find significant differences in *V. destructor* infestation levels in conditions of temperate semiarid and hot climates in the states of Zacatecas and Jalisco (Mexico), respectively.

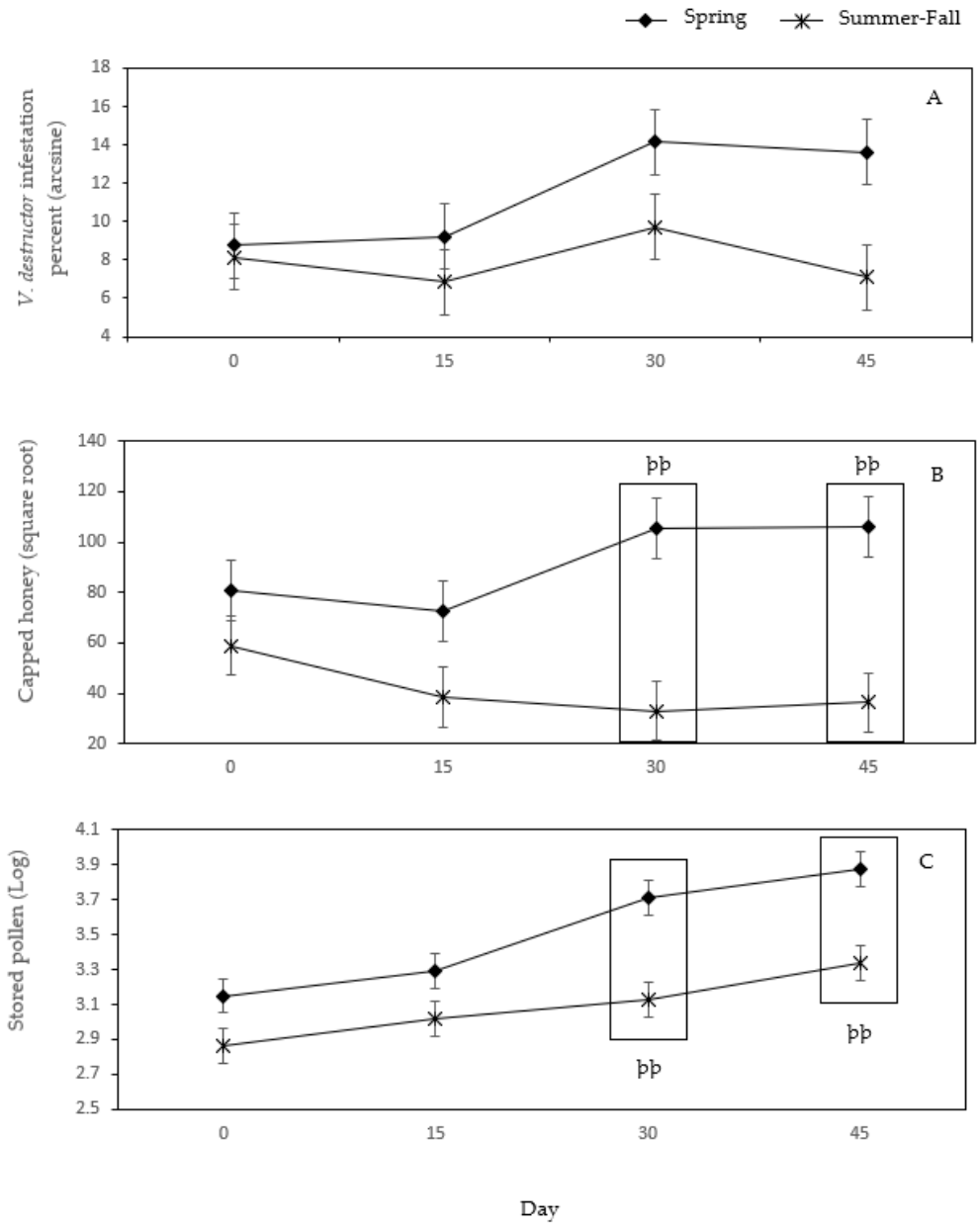

Figure 3.2 Behavior of honey and pollen reserves and level of *V. destructor* infestation during two flowering seasons (spring and summer-fall) in San Luis Potosí, Mexico. The values of the variables are presented in transformed units. þþSignificant difference at $p \leq 0.05$.

Regarding hygienic behavior (HB), a significant negative correlation (r = -0.65) was found with level of *V. destructor* infestation (Table 1). This coincides with results obtained by Masaquiza-Moposita *et al.* (2017), who found a negative correlation (r = -0.54) between HB and the number of mites parasitizing adult bees. In contrast, Strauss *et al.* (2015) found no correlation between the rate of brood removal and *V. destructor* infestation levels in colonies of *A. m. scutellata*, but

this correlation did exist with European *A. mellifera* hybrids. In this regard, Medina-Flores *et al.* (2014) mention that there are many inconsistencies among the results found in the literature on the degree of influence hygienic behavior can have on the level of *V. destructor* infestation, even when these studies have assessed this interaction in bee colonies selected for their expression of HB and fertilized artificially over generations. These authors attribute this variation in the results to a strong environmental influence that exists in the expression of hygienic behavior, as well as to methodological differences in the experiments.

When analyzing the effect of interaction of the flowering season in each sampling period, we observed that hygienic behavior had statistical difference only at the beginning of the experiment; it was higher in summer-fall, with a mean of 86.3% compared with 59.8% in spring. However, this difference was not maintained during the rest of the experiment (Figure 3c). Likewise, Medina-Flores *et al.* (2014) found similar percentages of HB in spring and fall, despite the important variation in the expression of hygienic behavior during the entire experiment.

In this sense, it is important to consider that HB plays an important role in the natural resistance of *A. mellifera* populations to *V. destructor*. Also, although the trait is genetically determined (Locke, 2016), environmental factors, as with the beekeeper's management techniques, can strongly affect its expression. Uzunov *et al.* (2014) studied 21 apiaries in different parts of Europe and 16 genotypes of different European subspecies of honeybees from 2009 to 2012 and concluded that the expression of HB was highly affected by harvest season and location of the apiaries, factors that interact to generate unique combinations of nectar and pollen availability and translate into widely diverse conditions of colony strength.

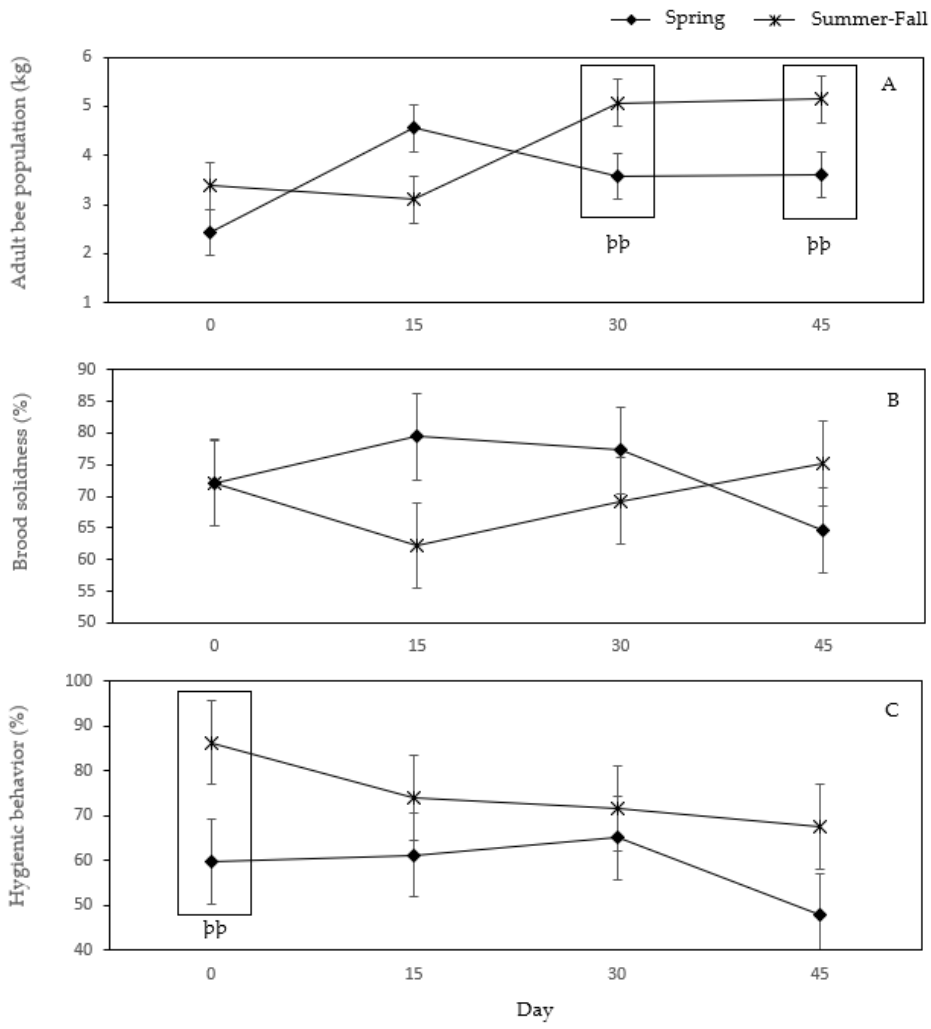

Figure 3.3 Behavior of the adult bee population, brood solidness, and hygienic behavior during two flowering seasons (spring and summer-fall) in two communities of San Luis Potosí, Mexico. The variables are presented in their original units. þþSignificant difference at $p \leq 0.05$.

It is not possible to speculate that the HB response is directly influenced by the strength of the population since there was no correlation with ABP. Moreover, the population had statistical differences as of the second half of monitoring; ABP was higher in summer-fall with 5.07 kg on day 30 and 5.14 kg on day 45, against 3.58 and 3.61 kg in spring, respectively (Figure 3a). In the same sense, a negative correlation was found between HB and the amount of capped honey (CH) in the brood chamber (r = -0.66). The amount of capped honey was statistically different between seasons as of day 30 of monitoring (Figure 2b); there was more in spring than in summer. This difference in the hive's honey storage capacity is possibly associated with a decrease in the foraging activity of worker bees derived from higher precipitation during the summer and thus fewer sunny days during the season. It has been shown that the presence of rains in the foraging

areas decreases traffic of pollinating insects by up to 30% (Linares *et al.,* 2021). It is also possible that there is a more abundant offer of nectar during the spring. This coincides with Medina-Flores *et al.* (2019), who assessed the same flowering seasons as in our study and observed a more abundant honey reserve during the spring than in the fall.

The abundance of reserves derived from a greater availability of nectar in the field could have limited the expression of HB and thus no differences were observed in the level of HB once the flowering seasons began. This phenomenon could be related to the distribution of tasks within the colony since the hygiene bees are able to interchange the activity of collecting nectar and hygienic behavior depending on the needs of the colony (Momot & Rothenbuhler, 1971). In contrast, Janmaat & Winston (2000) consider that the abundance of nectar and pollen is a factor that could motivate the expression of HB because it generates a greater need for storage space.

The brood solidness (BS) is a qualitative variable that can contribute to the evaluation of the health status of the colony. The results show that BS was equally consistent during both seasons (Figure 3b) and correlated positively with ABP (+0.40), which is reasonable since BS is an indicator of brood abundance and homogeneity, as well as of the egg-laying performance of the queen. A disperse pattern is sign of a problem in oviposition due to low sperm quality, egg death, etc. (EFSA-AHAW Panel, 2016), and it is an efficient predictor of the future adult population.

Regarding the variable stored pollen (POL), the colonies had statistical differences between seasons as of day 30 of monitoring (Figure 2c), and it was higher during the spring. At the same time, a significant correlation was observed between CH and POL (+0.65). This is logical since both products (nectar and pollen) are available in the field at the same time during flowering and are the mainstays for feeding the colony. The larger quantity of pollen stored by the colonies during the spring could be associated to the type of species that are flowering. Smart *et al.* (2017) mentions that even when the supply of plant resources in flower is highly diverse, the bees tend to concentrate their efforts on gathering a few species, either because the preferred species are more abundant or because they supply specific nutrients that the colony needs at a given moment. Therefore, although the summer season may offer a greater diversity, it is possible that the species of interest for the *A. mellifera* colonies were less abundant and, during the spring, the colonies showed greater preference for the species that were flowering and gathered a larger volume of pollen.

It is difficult to speculate on the positive correlation between the quantity of capped honey (+0.58) and pollen (+0.62) stored in the brood chamber and the level of *V. destructor* infestation. The level of infestation had only a slight numerical, non-statistical difference during the second half of monitoring, despite the significant increase in the quantity of honey and pollen reserves as of day 30. In contrast, the negative correlation observed between CH and the adult bee population (-0.56) is supported by the behavior of both variables. For example, during summer, the significant increase in population as of day 15 led to a significant reduction in the quantity of CH as of day 30 since the larger population consumes more food and therefore the reserves decrease (Medina-Flores *et al.,* 2019). In the same sense, this interaction between the quantity of CH and ABP could explain to a certain extent the correlations between VDIP and CH and

between VDIP and POL. Considering that the quantity of food reserves seems to be a good predictor of the size of the adult population, the reduction in the population derived from shorter longevity of the bees caused by *V. destructor* parasitosis during the larval stage (Aldea & Bozinovic, 2020) is reflected in the increase in food reserves.

The above makes it clear that the characteristics of some *A. mellifera* colonies that seem to have an influence on the mite populations may not have any influence in other colonies; the differences may be even more marked under different environmental conditions. However, the development and implementation of strategies aimed to monitor populations of this parasite, identify their economic thresholds, develop adequate prevention techniques, and apply treatments step by step in accord with the needs of the colony would allow us to reach an effective integral management strategy to deal with *V. destructor*.

3.5 CONCLUSIONS

Of the flowering seasons in the study area, summer seems to offer better conditions for the development of *A. mellifera* colonies, allowing significant growth of their population. Both the quantity of honey and pollen reserves and the adult population are the only variables associated with colony strength and seem to be affected by the flowering season, in such a way that the oviposition pattern will be determined by intrinsic factors likely associated with the reproductive performance of the queen. Moreover, we confirm that the expression of hygienic behavior is affected by a large number of factors and so can vary significantly over time and even within the same flowering season. Although it correlated strongly and negatively with the level of *V. destructor* infestation, it cannot be considered a reliable predictor of the population dynamics of the mite. Likewise, even when the quantity of food reserves has an important positive correlation with the variable of interest, it is not necessarily a causal relationship.

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4. ACARICIDE POTENTIAL OF CREOSOTE BUSH (*Larrea tridentata*) EXTRACTS IN THE CONTROL OF *Varroa destructor* IN *APIS MELLIFERA*

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4.1 ABSTRACT

To satisfy the growing need to assess the potential of natural compounds for the control of *Varroa destructor* in colonies of *Apis mellifera*, the acaricidal potential of the hydrolate and ethanolic extract (Leatricina®) of creosote bush (*Larrea tridentata*) was evaluated at a concentration 50%; contrasting them with the synthetic chemical amitraz and a control under *in vitro* conditions (48 h) and in the hive (52 days). There were differences ($p<0.05$) in *In vitro* Varroa mortality with amitraz with higher values (97.9%) compared with hydrolate (53.1%), and Leatricina® (51.5%); Creosote bush extracts were similar ($p> 0.05$). In the field experiment, the reduction in the percentage of varroa infestation in adult bees was higher 84.9 % ($p<0.05$) for amitraz, while the hydrolate and Leatricina® showed differences ($p<0.05$) 49.5 and 72% respectively, the control group showed the lowest values. Even when the effectiveness of synthetic chemicals presents an evident superiority, the average efficacy, and other benefits of natural compounds such as creosote bush extracts represent a viable and safe alternative for the control of *V. destructor*.

Keywords: *Varroa destructor*, *Larrea tridentata*, natural compounds, acaricidal potential.

4.2 INTRODUCTION

The ectoparasite *V. destructor* has represented one of the main pathological threats to the population of *A. mellifera* in the world [1] since its appearance and spread between 1950 and 1990 [2]. The process of coevolution between varroa and its original host, *Apis cerana*, allowed generating a stable relationship between them, thanks to the development of different

mechanisms of resistance and tolerance by the Asian honey bee [3]; even though *A. mellifera* shares some of these defense mechanisms, they are less accentuated [4], so the story told between the European honey bee and the varroa parasite has been different. The presence of varroa represents an important stress factor for the colony, since it feeds mainly to the adipose tissue of the bee, as recently demonstrated [5]; and reproducing inside the cells of bee brood, it significantly compromises their health. De Jong *et al.* (1982) [6] observed a decrease of 25% in the weight of parasitized bees at the time of emergence, in addition to negative effects on the longevity of adult bees, reducing their life expectancy by up to 50% [7]. The mite is associated with different viruses that can be lethal to honey bees, being the Deformed Wings Virus the most prevalent around the world due to its transmission and replication by varroa [8]; In addition, varroa has been identified as a relevant risk factor within the colony collapse disorder around the world [9]. There are some honey bees populations that survive to varroa [10], with the ability to maintain the mite population below permissible levels. It has been considered that some of the most important traits associated with this phenomenon are grooming and hygienic behavior sensitive to varroa [11], highlighting the importance of defense traits in the immunity of social bees [12].

The use of synthetic chemicals has been the method commonly used to control the varroa parasite. There are four active ingredients mainly used for this purpose, formamidine amitraz; the pyrethroids flumethrin and tau-fluavlinate; and the organophosphate coumaphos [13]. In Mexico, the continuous use of these products has produced populations of resistant mites. Rodríguez-Dehaibes *et al*. (2005) [14] estimated the increase of the LD_{50} of different acaricides over a period of 9 years and determined that in the case of amitraz this was 2.3 times higher, while for flumethrin the dose increased 327 times. Some years later, Rodríguez-Dehaibes *et al.* (2011) [15] again analyzed the level of varroa resistance against different acaricides in three beekeeping regions of Mexico, finding alarming results, especially in the region of Yucatan with a resistance index of 4057.32 for flumethrin, 199.57. for tau-fluvalinate, 26.55 for amitraz and 3.93 for coumaphos. The high resistance, mainly to the compounds of the pyrethroid group (flumethrin and tau-fluvalinate), possibly because both active ingredients were among the first chemical products available in the country for the control of varroa; gradually they lost popularity among beekeepers and were replaced by amitraz, due to its greater effectiveness. On the other hand, the risk of residuality of this type of compound in beehive products is something that must be considered since it represents a potential risk both for the bee colony and for human health. Orantes-Bermejo *et al.* (2010) [16] detected the presence of acaricides and pesticides in 100% of the wax and pollen samples analyzed, which presented contamination by 1 to 5 compounds, including the acaricide tau-fluvalinate. Similarly, Chauzat and Faucon (2007) [17] revealed the presence of tau-fluvalinate and coumaphos in 61.9 and 52.2 % of the wax samples analyzed; as well as Mullin *et al.* (2010) [18] showed contamination of 98% of their wax samples with tau-fluvalinate and coumaphos, as well as small amounts of amitraz.

Amitraz is a compound derived from formamidines that acts as an antagonist of octopamine receptors, which is a neurotransmitter or neuromodulator homologous to the noradrenergic system of vertebrates [19]; the effects of this acaricide are the hyperexcitability, paralysis and finally the death of the mite [20]. Amitraz is high effectiveness compared to other synthetic and organic acaricides [21, 22, 23]; however, the cost of products with this active ingredient specially formulated for its application in the hive (Apívar®) is high, so a big amount of beekeepers can´t afford it, and in many occasions they use products with the same active ingredient but that have been formulated for other domestic species and contain a higher concentration like Taktic® in the United States [24] or Bovitraz® in Mexico. Being an octopaminergic agonist in arthropods, there is a possibility that it also influences behavior, learning and formation of honey bees, also affecting some physiological processes related to various tissues and sensory organs [25].

Exposure to high doses of amitraz during the larval stage can lead to negative effects on their survival, decreased weight at the time of emergence, malformations in the antennae and hypopharyngeal glands, alteration in the gene expression of detoxification enzymes [26], decrease in the reproductive quality of drones by reducing their sperm viability [27], lower tolerance to viral diseases as well as alteration in their cardiac functions [28], alterations in the distribution of tasks inside the hive [29] as well as in the behavior during the foraging [30]; similarly, a higher mortality of adult bees has been observed in the week after the application of amitraz in the hive [31]. For all mentioned, many beekeepers oppose the application of synthetic chemicals, considering them harmful and unsafe for the hive; in response, different organic acids derived from active plant components have emerged, as well as essential oils that have shown to be effective for the control of varroa and present a low risk of accumulation or residuality in the products of the hive, in addition, not leading to the generation of resistance by the mite [32]. Among them, organic acids such as formic and oxalic acids stand out, as well as essential oils with thymol [13].

The creosote bush (*L. tridentata*) is a perennial shrub belonging to the Zygophyllacea family; it is widely distributed in Mexico, around of 25% of the desert areas in the states of Baja California North and South, San Luis Potosí, Coahuila, Durango, Zacatecas, and Nuevo León are covered with this shrub [33]. The creosote bush has been used for many years by tribes in North and South America for the treatment of multiple diseases due to its bactericidal, virucidal, and fungicidal properties and against internal and external parasites. Its use has been commonly given by means of aqueous and ethanolic extracts [34]. This species is a valuable source of secondary metabolites, considering that approximately 50% of the dry weight of the leaves is extractable material [35], where the resin is the main reservoir of compounds such as saponins, flavonoids, amino acids, minerals and mainly lignans. Phenolics, is the most prominent group of metabolites in relation to dry weight [36]. Nordihydroguayaretic acid (NDGA) is the best known and characterized lignan of this species, mainly for its antioxidant properties [35,37]. The physiological function of lignans is linked to defense activities against fungal and bacterial pests and diseases, as well as antioxidant and enzyme inhibitor [38]. The potential use of some lignans as insecticides has been recently

documented by obtaining results compared with the activity of synthetic pyrethroids [39]; Therefore, it is probable that the high content of phenolic lignans in creosote bush can provide important acaricidal properties to be used in the treatment of varroa. In addition to NDGA, the presence of other metabolites must also be considered, like more than 20 methyl aglycone flavonoids with numerous and varied effects. The combined activity of these constituents generates a synergism that amplifies the effect of the primary active compound (NDGA), suggesting the advantage of using an extract of the entire leaf/steam structure compared to using a purified or synthesized NDGA preparation [36]. Some extracts of *L. tridentata* like Leatricina® have been developed in our laboratory and quantitative analysis of individual bioactive components is described in López-Aguirre et al. (2016) [40].

To satisfy the need to generate viable organic alternatives for the control of varroa, of high availability and accessibility for the beekeeper, with low or null risk of toxicity for the hive and that don´t promote the generation of resistance by the mite; the aim of this study is to evaluate the efficacy of *L. tridentata* extract to control *V. destructor* in *A. mellifera* colonies.

4.3 MATERIALS AND METHODS

The present study was developed in two stages; the first, which we will call the *in vitro* phase, was carried out at the Instituto de Investigación de Zonas Desérticas, UASLP; the second stage or field experiment was carried out in the experimental apiary located in the town of San Elías, Armadillo de los Infante, S.L.P., Mexico; at the coordinates 22°18'49.50''N and 100°47'16.92''W.

4.3.1 Extracts

Two types of extracts were used, the first was the commercial product Leatricina®, (Nutrición y Genética Saludable S.A.de C.V. León, Gto. Mexico) ok which is an ethanolic extract based on *L. tridentata*. The second was a hydrolate from *L. tridentata* obtained by steam stripping technique. The collection of the plant was carried out in April and May 2022, in the days after the first spring rains and in the early hours of the morning. Samples of stems with leaves, flowers and fruits were collected, these were transported in black polyethylene bags to the phytochemistry laboratory of the Instituto de Investigación de Zonas Desérticas, UASLP. From the collected samples, mainly leaves, flowers and fruits were selected until completing 900g, these were placed in a round-bottomed boiling flask with a capacity of 1000 ml and 200 ml of water were added; Subsequently, the flask with plant material was mounted in a rustic model of simple distillation that consists of the application of steam directly to the plant material, to obtain a mixture of steam with essential oil, which will be dragged to a refrigerant system to achieve its condensation and obtain hydrolate. The distillation process lasted four hours, at a temperature of 92°C. Both extracts were diluted with distilled water to reach the concentration for each treatment.

4.3.2 In vitro test

Previously, with the purpose of evaluating the toxicity of creosote bush extracts on bees, treatments of 12.5, 25, 50 and 100% concentration of each one of the extracts were applied to groups of 10 bees in experimental cages, each cage was considered a replicate and four replicates per dose or treatment were performed. The cages were placed in an incubator for 48 h at a temperature of 34 °C and a relative humidity of 65 % to simulate the environmental conditions inside the hive, the bees were fed honey *ad libitum* and water was supplied three times a day. No bee mortality was recorded in any of the treatments during the 48 h of observation, so a possible toxic effect on adult bees was discarded.

To evaluate the acaricidal potential of creosote bush extracts, samples of approximately 200 parasitized adult bees were collected, these were taken directly from the frames of the brood. The hives has Italian queens and were in the experimental apiary and presented a high percentage of varroa infestation. The sample of bees was placed in small plastic cages (20 x 20 x 10 cm) with holes at the top to allow oxygen to enter and fitted with a plastic mesh (3.5 mm) at the bottom to facilitate the passage of the mites, but not the bees. Additionally, a removable bottom was placed at the bottom of the cages with a depth of 1.5 cm covered with Vaseline, so that the mites that fell to the bottom were stuck and couldn´t escape. Each group of bees was sprayed with approximately 1.5 ml of the corresponding extract, and in the case of treatment with amitraz, a 2.5 g strip fragment of Apivar® was placed inside the cage. Subsequently, they were placed in an incubator at 34°C and a RH of 65%. Observations were made at 1, 2, 4, 8, 12, 24 and 48 h, checking the removable bottom of the cages to count the dead mites, verifying with a magnifying glass the lack of response and mobility by the mite to an external stimulus. At the end of the observation period, all the bees were sacrificed using the method described by De Jong *et al.* (1982) [6], which allowed counting the mites that were still attached to the body of the bees and determining the initial level of infestation of the sample using the following equation:

$$Infestatión\ percentage\ = \frac{Amount\ of\ mites\ in\ the\ sample\ x\ 100}{Amount\ of\ bees\ in\ the\ sample}.$$

4.3.3 Field experiment

For the evaluation of the efficacy extracts of *L. tridentata* in the hive, four treatments were established: Leatricina® 50%, hydrolate 50%, amitraz and a control with four replicates for each one, each hive being considered as a replicate. The concentration percentage of the extracts was selected based on the results of the *in vitro* test. Jumbo hives of 9 to 10 frames (2 with food reserve and 7-8 of brood) with commercial Italian queens were used, all the test hives were statistically similar in the percentage of varroa infestation. The nutritional requirements of the hives were covered by the natural flowering present in the site, which did not present a sufficient nectar flow to obtain a harvest.

The field experiment lasted 52 days starting on August 23, 2022; during the first 16 days the natural mortality of varroa was monitored (figure 1). The applications of the *L. tridentata* extracts were made every eight days. For the application, an atomizer was used to spray the product finely and uniformly directly on the frames covered with bees, using an approximate quantity of 50

ml/hive in each application. For the treatment with amitraz, two Apivar® strips were placed between the third and fourth frame, and between the seventh and eighth frame. These were removed on 49 days. Finally, to evaluate the efficiency of each treatment and quantify the remaining varroa population in each colony, a standard treatment of Apistan® (tau-fluvalinate) was applied to all test hives by placing two strips of the product in each box on 49 days.

Figure 4.1 Schedule of treatments application in the hive.

The percentage of varroa infestation was determined using the method described by De Jong *et al.* (1982) [6]. Three samplings were carried out using this methodology, before starting the treatment, at the end of the application of the treatment, and after the application of the standard Apistan® treatment. Varroa mortality was estimated by placing 52 x 35 x 1.5 cm wooden trays at the bottom of the hive, covered by a plastic mesh that allowed the passage of the mites that fell to the bottom, but prevented the passage of the workers. A sheet of aluminum foil covered with vegetable oil was placed on each tray so that the mites would remain stuck once they fell. The tray was removed every four days to remove the sample and replace the aluminum foil with a new one. The aluminum foil was placed in a plastic bag to later count the dead mites in the laboratory. The mite population during the first 16 days of monitoring the natural mortality of the mite was estimated with the help of the equation proposed by Jack *et al.* (2019) [41], where *y* corresponds to the total number of mites present in the sample, then *x* is divided by the number of days that the sample remained in the hive, as shown below:

$$x = \frac{3.76 - y}{-0.01}.$$

4.3.4 Experimental design and statistical analysis

During the *in vitro* test, 11 treatments distributed as follows: concentration levels of 25, 50, 75 and 100 % for the hydrolate (H25, H50, H75 and H100) and Leatricina® (L25, L50, L75 and L100) were established; a treatment with the synthetic chemical acaricide amitraz (AM), a group with 96% ethanol to discarded possible effects as part of the ethanolic extract (ET) and a control group (CO). Four replicates per treatment were carried out in a completely random design, collecting information of the number of dead mites in each sequenced observation at 0, 1, 2, 4, 8, 12, 24 and 48 h, which were analyzed by means of an orthogonal polynomial test and an analysis of covariance for the variable "decrease in the percentage of varroa infestation", which was transformed into arcsine to achieve the assumption of normality. The “initial infestation

percentage” in each replicate (plastic cage) was used as a covariate. In addition, an analysis of variance with repeated measures was carried out for the variable "percentage of live mites", which was also transformed into arcsine.

In the field experiment, four treatments were established: Leatricina® 50% (L50), hydrolate 50% (H50), amitraz (AM) and a control (CO), each treatment had four replicates under a completely random design. The variable "percentage of infestation in adult bees" was transformed into arcsine for analysis of variance with repeated measures. The variable "mortality of *V. destructor*" was transformed using the Johnson transformation tool in Minitab software, the function generated for the best data fit was:

$$1.37593 + 0.603408 \times \text{Ln}((X + 3.10188) / (987.292 - X)),$$

subsequently, an analysis of variance with repeated measures was applied to the transformed data. Finally, an analysis of variance with a with a randomized complete block design to the "relative efficacy" in vitro and in the field was made, data expressed as a percentage. For data analysis, the SAS OnDemand for Academics: Studio software was used.

4.4. RESULTS

4.4.1 In vitro test

The nature of the response of Leatricina® to different concentrations showed a quadratic effect (figure 2), increasing progressively until reaching the best result at a concentration of 75% and subsequently its effect decreases at higher concentrations. On the other hand, the effect of the *L. tridentata* hydrolate evolves favorably as its concentration increases until reaching 50%, subsequently the effect seems to decrease even despite the increase in the concentration of the product until 75%, at higher concentrations we can observe a favorable response, presenting a behavior of cubic type.

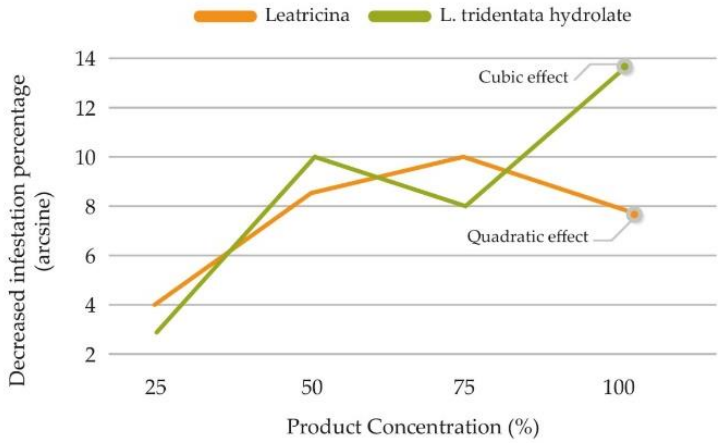

Figure 4.2 Behavior of the decrease in the percentage of infestation of *V. destructor* when applying Leatricina® and *L. tridentata* hydrolate in different concentrations.

The effect of the extracts of *L. tridentata* at different concentrations and the synthetic chemical amitraz on the reduction of the percentage of infestation of varroa at the *in vitro* level in shown in figure 3. Amitraz was the best product for varroa control ($p<0.05$), while the treatments with hydrolate and Leatricina® at concentrations of 50, 75 and 100% show a similar behavior. The treatment with hydrolate and Leatricina® 25% presented the least effectiveness *in vitro* and a similar response to ET and CO.

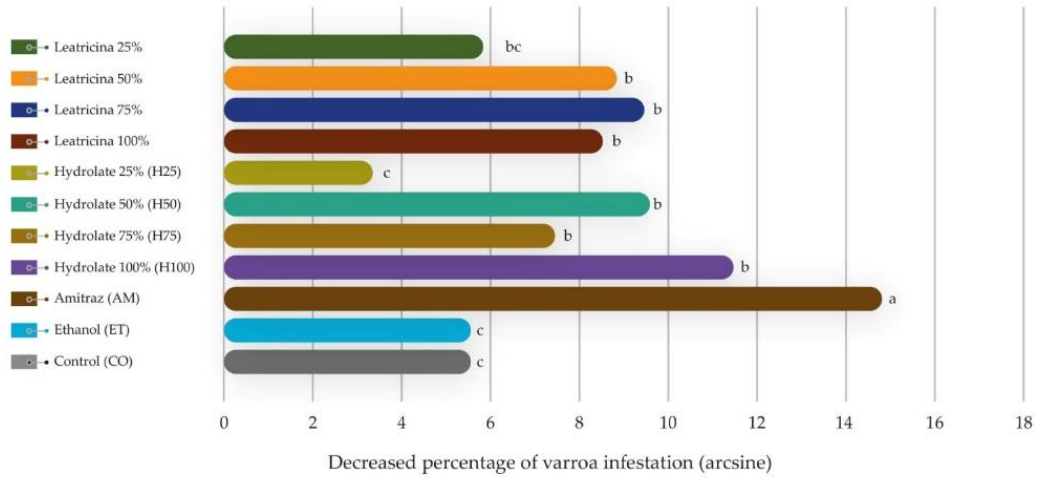

Figure 4.3 Decrease in the percentage of infestation *in vitro* of *V. destructor* applying extracts of *L. tridentata* at different concentrations and the synthetic chemical amitraz. ^{a,b,c} means with different letter in a column are different ($p<0.05$)

Evaluating the survival of *V. destructor* mites in a 48-h period applying only the treatments proposed to be taken to the field experiment, statistical differences were observed after the second hour (figure 4). The AM treatment presented lower survival ($p<0.05$), eliminating 97.9% of the mites present in the sample. The L50 treatment presents the second-best response, which is not comparable to the AM synthetic chemical, but is statistically superior to CO and ET after two hours, when the effect of the extract seems to end, during the following hours mite mortality doesn´t increase significantly and practically remains the same, finally eliminating 51.6% of the mites present. H50 shows a longer effect than L50, possibly because Leatricina® is an ethanolic extract and its evaporation is faster. Hydrolate 50% shows a similar effectiveness to CO and ET during the first 4 hours, from this moment on, a higher effectiveness than the control treatment is observed, eliminating 53.1% of the mites present. Regarding the control treatment, like ET, it maintains a 100% survival of the mites during the first 8 hours. The natural mortality of the mites occurs after 12 h, with a final survival of 81.6% in CO and 82.6% in ET.

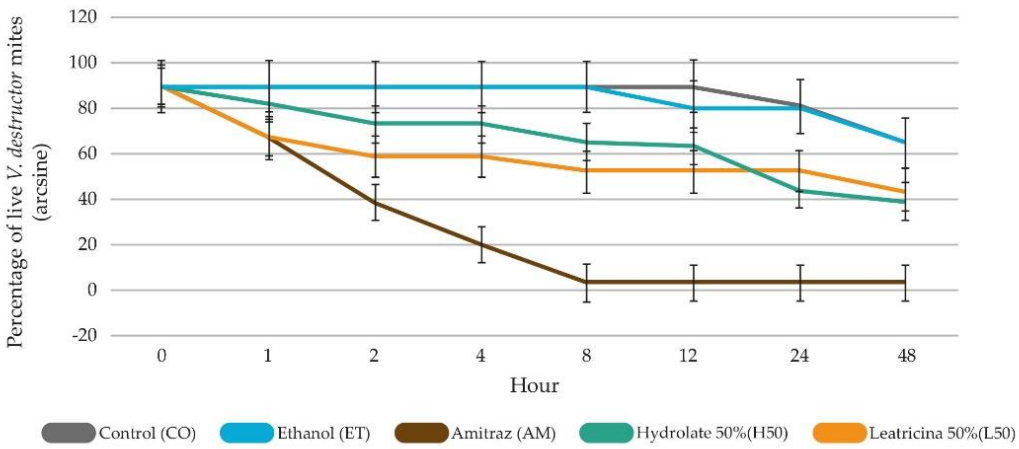

Figure 4.4 Behavior of mortality *in vitro* of *V. destructor* mites during a period of 48 h with the application of *L. tridentata* extracts and the synthetic chemical amitraz.

4.4.2 Field experiment

Prior to the application of the treatments (day 16), the percentage of infestation in the hives of all the treatments was homogeneous and didn´t present statistical differences, showing values of 6.9% for CO, 8.8% for AM, 6.4% for H50 and 10.1% for L50 (figure 5). At the end of the period of application of the treatments, AM presented the best performance ($p<0.05$), significantly decreasing the percentage of varroa infestation until 3.2%. On the other hand, the percentage of post-treatment varroa infestation in the hives treated with L50 decreased to 7.6%; however, this result wasn´t like that obtained by AM. Contrary to the rest of the treatments, CO and H50 presented an increase in the percentage of infestation, with values of 11.9% and 9.1% respectively.

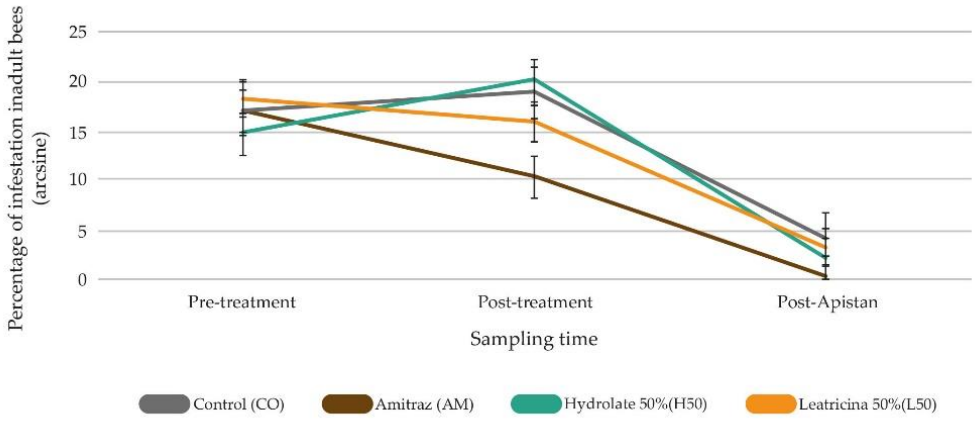

Figure 4.5 Behavior of the infestation levels of *V. destructor* in adult bees with the application of different extracts of *L. tridentata* and the synthetic chemical amitraz, at different times of the experiment.

The natural mortality of varroa during the first 16 days of monitoring was statistically similar among all the hives in the experiment, with an average mortality of 38.7 mites/day, which according to the formula proposed by Jack *et al.* (2019) [41] is equivalent to an approximate population of 3,494 mites. The control treatment behavior was statistically similar during the 48 days of sampling except for day 24, where there was an increase in mite mortality (figure 6). In addition, CO did not present statistical differences with the records of natural mortality (0-16 days) of the rest of the treatments. For AM, the highest mortality was recorded on 20 days, immediately after placing the amitraz strips inside the hive, being statistically higher ($p<0.05$) than the rest of the treatments. The mortality in AM treatment decreased significantly at 28 days, even below natural mortality, this due to the drastic reduction of the mite population in the hives treated with this synthetic chemical. The behavior of varroa mortality in hives treated with L50 remained constant until 36 days, from which time it decreased significantly to values like those presented by AM. Finally, the mortality in the H50 treatment didn´t present changes in mortality during all the sampling period and statistically it was the treatment more like the control.

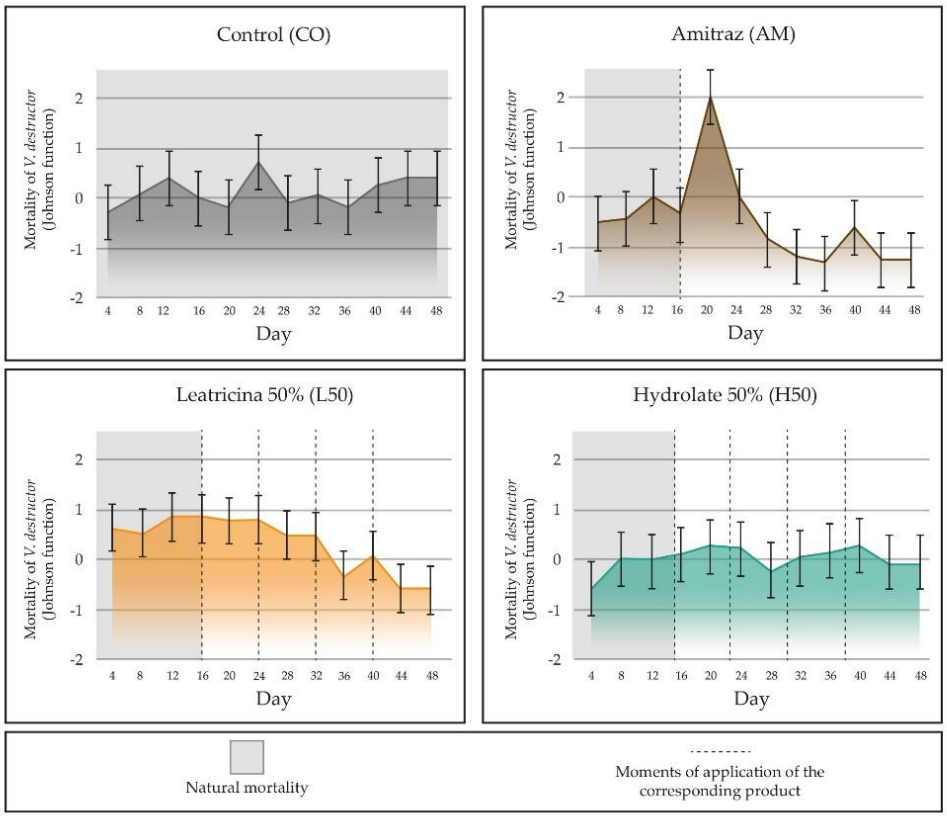

Figure 4.6 Behavior of mortality of *V. destructor* in the hive during a period of 48 days applying extracts of *L. tridentata* and the synthetic chemical amitraz.

Amitraz presented the highest values ($p<0.05$) of efficacy, with 97.9% *in vitro* conditions to 84.9% in the field experiment (table 1). The relative efficacy of the extracts of creosote bush were statistically similar, except for H50 in the field experiment. The hydrolate 50% varied from 53.1% (*in vitro*) to 49.5% in the field, while the Leatricina® 50% seems to be favored by field conditions (72%) with respect to the *in vitro* test (51.6%). The efficacy of the extracts of *L. tridentata* (hydrolate 50% and Leatricina® 50%), is good since the *L. tridentata* extracts are natural compounds and don´t present negative effects as synthetic chemicals. Finally, the control shows that the natural mortality of *V. destructor* mites varies from 18.3% under *in vitro* conditions to 47.4% in the field, presenting an important difference.

Table 4.1 Relative efficacy of extracts of *L. tridentata* and the synthetic chemical amitraz.

Treatment	Relative efficacy (%)	
Control	35.8 ± 29.97	b
Amitraz	92.3 ± 11.94	a
Hydrolate 50%	51.6 ± 30.17	b
Leatricina® 50%	63.3 ± 25.54	ab

[a,b,c] means with different letter in a column are different ($p<0.05$).

4.5 DISCUSSION

The effectiveness of the chemical amitraz in the present study was superior in all cases, proving to be effective in reducing the level of varroa infestation and presenting high percentages in mite mortality; this is similar to was observed by Gregorc *et al.* (2018) [21] who registered a mortality of 98.2% of mites during the first 6 h *in vitro* and 82% mortality of varroa in the field experiment, compared with the natural compound thymol (78 to 85%). In the same sense, Al Naggar *et al.* (2015) [22] obtained an efficacy of 76.5% when using amitraz for the treatment of varroa-infested hives in Canada compared with thymol (26.7%); similarly, they observed a higher percentage of winter survival in the colonies treated with amitraz (93%) than with thymol (67%).

Although the synthetic chemical amitraz is an effective treatment for the control of varroa, the use of *Larrea tridentata* extracts is a good alternative as shown in the present study, particularly L50 when is applied in the hive, showing an efficacy close to that of amitraz. It is necessary to consider that the efficacy of natural and synthetic acaricidal compounds is highly variable and their response is influenced by the environmental conditions of the scenarios in which they are applied. Gregorc *et al.* (2018) [21] observed that when they used in the field two natural acaricides and two synthetic chemicals (amitraz and HopGuard®; thymol and tau-fluvalinate) didn´t find statistical differences in their efficacy. On the other hand, Gracia *et al.* (2017) [42] didn´t observe a difference in the field efficacy of the synthetic chemical amitraz and the natural products Api Life Var® (thymol, eucalyptus, menthol, and camphor), thymol dissolved in oil and thymol dissolved in alcohol (home preparations). They consider that even when the formulation

of the products seems to be one of the most relevant factors regarding their effectiveness, the variation in the results depended on the conditions of the apiary in which they are applied. Carmona *et al.* (2002) [43] mention that even when the application of treatments for varroa control is recommended during spring and autumn, applications at the end of autumn or close to winter are ineffective, since parasitism is higher in the brood than in adult bees and may cause a decrease in the effectiveness of acaricides; this is because the dynamics of the varroa population is influenced by the dynamics of the *A. mellifera* colony, especially by the availability of brood. Maya-Martinez *et al.* (2020) [44] demonstrated that during the periods close to the hibernation season of the colony, the amount of bee brood is gradually reduced, therefore the reproduction opportunities for varroa mites also decrease, generating a higher concentration of mites inside the cells fulfilling their reproductive functions and reducing the percentage of mites parasitizing adult bees. Thus, the efficiency of the treatments for the control of varroa is greater when applied during seasons of high amount of brood of *A. mellifera* compared to winter, since the effect of the compounds occurs mainly on the mites that parasite adult bees and not on those found in the brood.

The *in vitro* and field efficacy of creosote bush extracts from this study for the control of varroa show encouraging results on the acaricidal potential of this plant species, since its effect is like to that observed with the use of some organic options available in the market. For example, Gregorc *et al.* (2018) [21] obtained similar results in the mortality of varroa mites when using the organic product thymol under *in vitro* conditions, observing a mortality of 33 to 34% with a 24-h action time. Cameron and Ellis (2021) [13], when carrying out an exhaustive review of the natural chemical compounds commonly reported in the literature for varroa control, classify thymol and hop beta acids as moderately effective products with 25 to 75% efficacy, and the results of mortality with the use of formic acid varies from 35 to 75%. Qadir *et al.* (2021) [45] reported an efficacy of 54.13% using 65% formic acid to reduce the varroa mite population. Ardeshir *et al.* (2002) [46] obtained 43 to 58% mortality of varroa mites when spraying solutions with 2% essence of thyme, mint, and dill on parasitized bees. So, the effectiveness of the extracts of creosote bush is within the ranges of efficacy considered for this type of products of natural origin.

Saldívar (2003) [47] reveals that when using extracts of *L. tridentata* at an *in vitro* condition, they can inhibit the proliferation of some insects. Mainly due to its high lignan content, especially nordihydroguayaretic acid, which is associated with plant resistance against pests and diseases [38]. On the contrary side, Viglianco *et al.* (2006) [48] consider that the potential of creosote bush it's in the repellent and anti-food effect. They observed that the ethanolic extract of *Larrea divaricata* leaves has a degree of repellency of 40 to 60% when used against *Sitophilus oryzae*. As observed in the present study with the application of the L50 ethanolic extract in the field and the significant reduction in varroa mortality from day 36, indicating a significant decrease in the mite population without a previous massive event of mite mortality, being able to attribute this phenomenon to a repellent effect that displaced a significant percentage of the mite population out of the hive. Marín-Domínguez *et al.* (2014) [49] observed a certain degree of repellency (35 to 40%) when using creosote bush aqueous and methanolic extracts on *Melanocallis caryaefoliae*. Maldonado-Simán *et al.* (2018) [50] observed a reduction of up to 68% in the count of horn flies (*Haematobia irritans*) perched on cows sprayed with aqueous extract of *L. tridentata*. Although these percentages may seem low and insufficient when compared to synthetic

chemicals such as amitraz. Malik *et al.* (2007) [51] proposes that the viability of using any natural compound as a pesticide should be considered if its efficacy is between 30 to 85%, considering that the availability of plant material is high and that this type of compound is environmentally friendly. In addition, it should be taken into account that, just as it is common to find a wide variation in the results of the application of synthetic chemicals because they are applied to the colonies in a different way from each other due to their varied nature and formulations, as well as the restrictions of use indicated in the labeling, the variation that we can observe when using natural compounds is even greater [13].

4.6 CONCLUSIONS

Despite the undeniable superiority of synthetic chemicals such as amitraz in the control of *V. destructor*, the results of the present study show the great potential of *L. tridentata* extracts, which, in addition to their moderate efficacy in the control of varroa, do not represent a risk of toxicity for adult bees or human health, so it could be used as a prophylactic treatment at any time of the year; its elaboration is low cost and easily reproduced by the producer, in addition to the high availability of plant material for its elaboration. It's recommended continue with the investigation of the effects of other types of creosote bush extracts, application methods, as well as their assessment under different environmental conditions, and the effect of this type of products on the larval stage of the bees; all the above with the objective of consistently defining the benefits and limitations of the use of *L. tridentata* in the control of *V. destructor* as part of a sustainable production strategy.

Author Contributions: Conceptualization, J.C.G.-L. and R.E. H.-M; methodology, J.C.G.-L. and R.E. H.-M; software, G.Á.-F.; validation, J.C.G.-L., R.E.H.-M., C.C.-S. and G.Á.-F.; formal analysis, R.E.H.-M., J.C.G.-L., G.Á.-F. and C.C,-S.; investigation, R.E. H.-M. and J.C.G-L.; resources, G.Á.-F., C.C.-S. and J.C.G.-L.; data curation, R.E.H.-M. and J.C.G.-L.; writing—original draft preparation, R.E.H.-M., G.Á.-F., C.C.-S. and J.C.G.-L.; writing—review and editing, R.E.H.-M., G.Á.-F., C.C.-S. and J.C.G.-L.; visualization, J.C.G-L.; supervision, J.C.G.-L.; project administration, J.C.G.-L.; funding acquisition, J.C.G-L. All authors have read and agreed to the published version of the manuscript."

Funding: This present research was financed by the support of Proinnova-CONACYT, Mexico-Nutrición y Genética Saludable, Léon, Gto. Mexico. Agreement number: 251911.

Informed Consent Statement: "Not applicable"

Conflicts of Interest: "The authors declare no conflict of interest."

4.7 REFERENCES

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CONCLUSIONES GENERALES

El parásito *Varroa destructor* representa uno de los principales problemas sanitarios para la apicultura de la región. Aun cuando en su mayoría las estrategias para su control se sustentan en el uso de químicos sintéticos como el amitraz, cuya efectividad ha permitido mantener los porcentajes de infestación de varroa en la región dentro del umbral económico, existe un creciente interés en los apicultores locales por descartar el uso de químicos sintéticos y optar por el uso de alternativas naturales.

La gobernadora (*Larrea tridentata*) es un recurso local altamente disponible en las zonas donde se desarrolla la apicultura, la cual además de su amplia gama de propiedades ya documentadas, posee un potencial moderado para el control del ácaro varroa sin los inconvenientes presentado por el uso de acaricidas sintéticos. Al no representar un riesgo para la salud de la propia colonia de *A. mellifera* o el consumidor, puede ser empleada en cualquier etapa del ciclo productivo de la colmena como un tratamiento profiláctico o como parte de una estrategia integral de manejo en el apiario. El presente proyecto de investigación solo es el primer paso hacia el descubrimiento del gran potencial que posee *L. tridentata* para ser incorporada dentro del manejo de la colmena.

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