Acaricide potential of Creosote Bush (*Larrea tridentata*) extracts in the control of *Varroa destructor* in *Apis mellifera*

Abstract: The aim of the present study was to assess the potential of natural compounds for the control of *Varroa destructor* in colonies of *Apis mellifera*, the antimite 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 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 withhydrolate (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.

1. 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 for a stable relationship to be generated, 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 honeybee and the varroa parasite has been different. The presence of varroa represents an important stress factor for the colony since it feeds mainly onthe 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 honeybeespopulations that surviveto 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, 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 insecticideshas 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 generatesa 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.

2. 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 Infantes, S.L.P., Mexico; at the coordinates 22°18ˈ49.50ˈˈN and 100°47ˈ16.92ˈˈW.

2.1 Extracts

Two types of extracts were used, the first was the commercial product Leatricina®, (Nutrición y GenéticaSaludable 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.

2.2 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 hasItalian 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 and 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:

$$Infestatión\ percentage\ = \frac{Amountofmitesint\quad hesamp\ lex\ 100}{Amountofbeesint\quad hesample}.$$

2.3 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 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 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:

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

2.4 Experimental design and statistical analysis

During the *in vitro* test, 11 treatments were 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, 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 control (CO), each treatment had four replicates. 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:

$$1.37593 + 0.603408 \times \ln((X + 3.10188) / (987.292 - X)),$$

Subsequently, an analysis of variance with repeated measures was applied to the transformed data. Finally, an analysis of variance with a 2x4 factorial arrangement was applied to the "relative efficacy", data expressed as a percentage. For data analysis, the SAS OnDemand for Academics: Studio software was used.

3. Results

3.1 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 the cubic type.

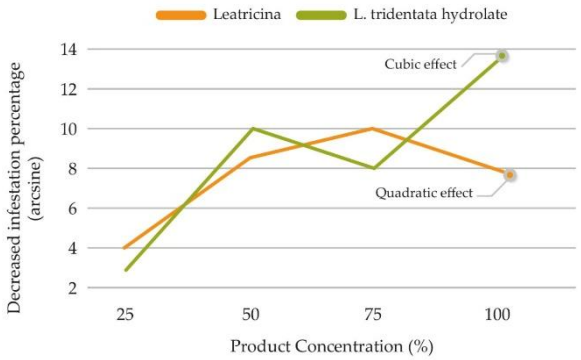

Figure 2. The behavior of the decrease in the percentage of infestation of *V. destructor* when applying Leatricina® and *L. tridentata*hydrolate in different concentrations.

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% showed similar behavior. The treatment with hydrolate and Leatricina® 25% presented the least effectiveness *in vitro*and a similar response to ET and CO.

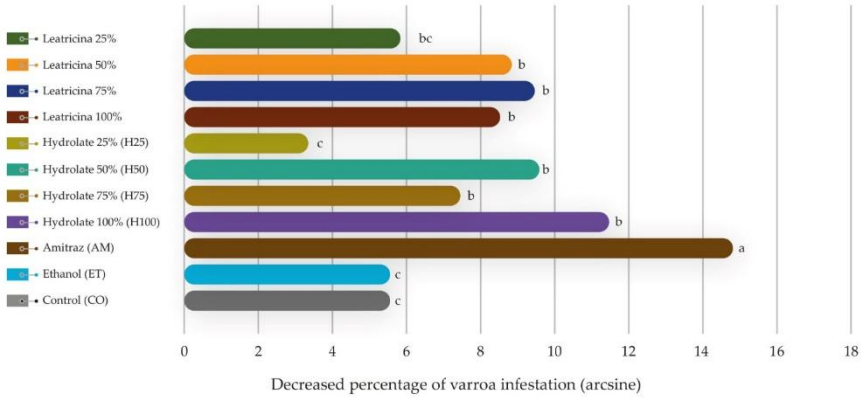

Figure 3. Decrease in the percentage of infestation *in vitro*of *V. destructor*by applying extracts of *L. tridentata* at different concentrations and the synthetic chemical amitraz.[a,b,c] means with different letter in a column are different ($p<0.05$)

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.

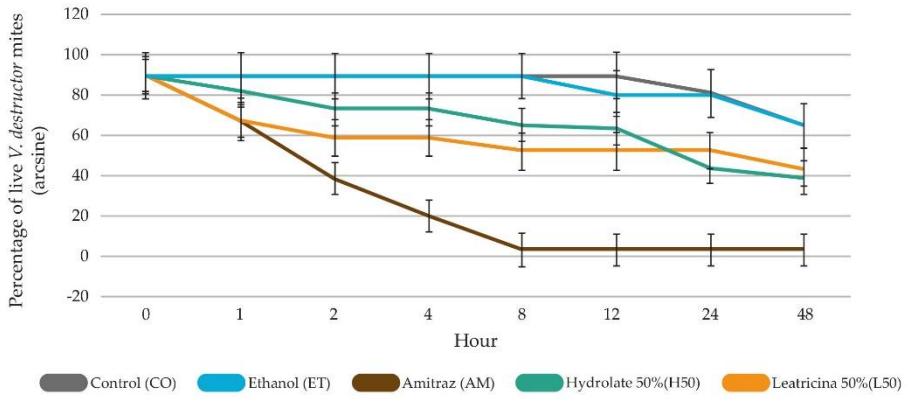

Figure 4. Behavior of mortality *in vitro* of *V. destructor* mites during a period of 48 h with the application of *L. tridentata* extracts and the synthetic chemical amitraz.

3.2 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.

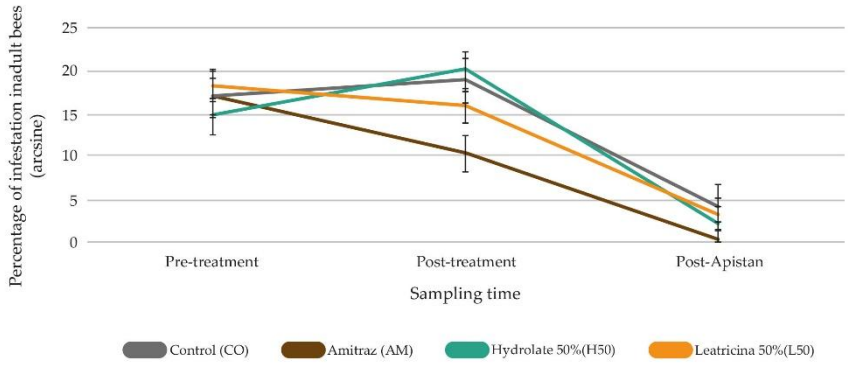

Figure 5. Behavior of the infestation levels of *V. destructor* in adult bees with the application of different extracts of *L. tridentata* and the synthetic chemical amitraz at different times of the experiment.

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, 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 periods, and statistically, it was the treatment that was morelikethe control.

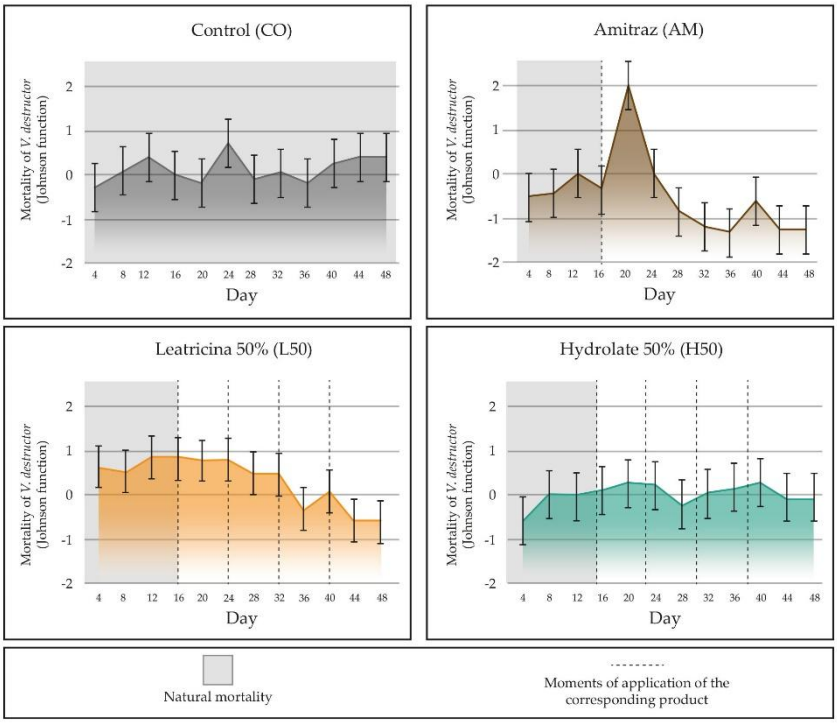

Figure 6. Behavior of mortality of *V. destructor* in the hive during a period of 48 days applying extracts of *L. tridentata* and the synthetic chemical amitraz.

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 1. Relative efficacy *in vitro* and in field experiment of extracts of *L. tridentata* and the synthetic chemical amitraz.

Treatment	Relative efficacy (%)			
	In vitro		**Field experiment**	
Control	18.3	c	47.4	c
Amitraz	97.9	a	84.9	a
Hydrolate 50%	53.1	b	49.5	c
Leatricina® 50%	51.6	b	72.0	b

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

4. 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 what 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), theydidn´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 thatduring 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 thatparasite 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 product 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 *Melanocalliscaryaefoliae*. Maldonado-Simán*et al.* (2018) [50] observed a reduction of up to 68% in the count of horn flies (*Haematobiairritans*) 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].

5. 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 that 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 to 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.

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