Article

Presence of *Varroa destructor*, *Nosema* spp. and *Acarapis woodi* in colonies of bees from Tabasco, Mexico

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Abstract

Bee (*Apis mellifera*) diseases cause great economic losses and are considered the main cause of the high loss of colonies. In this study, the frequency and infestation levels of *Varroa destructor* and *Acarapis woodi* were determined. Likewise, the frequency and infection levels of *Nosema* spp. in colonies of honeybees from Tabasco, Mexico, were determined. During October to November 2020, bee samples were collected in 112 colonies in the five subregions of Tabasco. The frequency and levels of infestation of *V. destructor* were diagnosed using the capped brood comb fragment technique, as well as the washing of adult bees. The quantification of spores of *Nosema* spp. was performed by macerating abdomens and visual identification of *A. woodi* in tracheas using a stereoscopic microscope. At the state level, it was found a frequency of *V. destructor* in capped brood (FVCO)= 69.64%; level of infestation of *V. destructor* in capped brood (NIVCO)= 13.86% (p= 0.419), likewise, it was observed a frequency of *V. destructor* in adult bees (FVAA)= 92.86%; level of infestation of *V. destructor* in adult bees (NIVAA)= 3.88% (p= 0.944). It was observed a frequency of *Nosema* spp. (FN)= 91.96%; infection level of *Nosema* spp. (NIN)= 133 738 ±156 221 spores/bee. All samples were negative for *A. woodi*. The results indicate that the presence of *V. destructor* and *Nosema* spp. are more frequent in the subregions studied, the levels of infestation of *V. destructor* and the levels of infection of *Nosema* spp. are low. However, extreme values that exceed the tolerable limits were found in various communities, which could represent foci of attention before possible dissemination of these pathogens.

Keywords: *Apis mellifera*, *Acarapis woodi*, *Nosema* spp., *Varroa destructor*.

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Introduction

Worldwide, Mexico ranks sixth among the countries with the highest honey production, ranking third in the Americas, after the United States of America and Argentina; likewise, it is among the main honey-exporting countries with 23 768.84 t with a value of $ 57 576 183 USD, with the main importing countries being Germany (1 481.6 t), the United States of America (2 567.98 t), the United Kingdom (1 992.58 t), Saudi Arabia (1 481.6 t), Switzerland (1 440.78 t) and Belgium (1 212.9 t) (FAO-FAOSTAT, 2021).

According to information obtained from SIAP (2021), the main honey-producing states in Mexico are Yucatán (10 022 t), Campeche (8 797 t), Jalisco (6 138 t), Chiapas (5 483 t) and Veracruz (4 891 t); while the state of Tabasco ranks 25th in honey production (408 t). In addition to their social and economic importance, bees pollinate 77% of the plants responsible for producing the food resources that sustain the world's human population (Buchmann and Nabhan, 1996), so FAO (2021) has considered that beekeeping impacts 10 of the 17 objectives of the 2030 agenda for sustainable development.

In recent years worldwide, beekeeping has been threatened by climate change, changes in land use, indiscriminate use of pesticides and by the high loss of colonies, which is considered to be the result of many factors within which various pathological agents are involved (Roy *et al*., 2018). Therefore, studies related to the impact of beekeeping on the economy in various countries and comprehensive studies to determine the health status of colonies are relevant at the local level. Varroosis is a parasitosis caused by the mite *V. destructor* in *A. mellifera* and is considered the main health problem that affects beekeeping worldwide, in addition to favoring the proliferation of other microorganisms such as *Nosema* spp.

Various researchers such as Van Engelsdorp *et al*. (2008); Paxton (2010) reported that *Nosema* spp. is widely distributed in North America and Europe, where the phenomenon called colony collapse disorder (CCD) has drastically caused the death of hundreds of thousands of colonies, so these backgrounds evidence *Nosema* spp. as a possible causative agent. Recently, Martínez and Cetzal (2018) reported that *Nosema* spp. has markedly increased its frequency and levels of infection in Yucatán, Mexico. Several researchers have proposed that *N. ceranae* is a new parasite of *A. mellifera*, of which much is still unknown about its pathology and how it may interact with other stressors (Guerrero and Molina *et al*., 2016; Roy *et al*., 2018).

In South America, various studies such as those conducted by Klee *et al*. (2007) in Brazil, Invernizzi *et al*. (2009) in Uruguay, Medici *et al*. (2011) in Argentina and Martínez *et al*. (2012) in Chile, have confirmed the presence of *N. ceranae*. On the other hand, in Mexico, the detection of *Nosema* spp. was carried out in 2011, with samples collected since 2004 (Guzmán *et al*., 2011). In recent years, various works have been carried out to detect the presence of *Nosema* spp., both in commercial colonies and wild swarms, as evidenced by the works carried out by Casavantes (2011); Acosta *et al*. (2014) in Chihuahua; Martínez *et al*. (2011) in Yucatán, as well as in Campeche, Martínez and Catzín (2012) reported a high frequency of *Nosema* spp. (99%) in the samples collected, concluding that Nosemosis has been increasing over the years.

On the other hand, the tracheal mite *A. woodi* is an obligate internal parasite, which mates and develops within the tracheas of the respiratory system of adult bees, it is microscopic, feeds on the hemolymph of honeybees, causing significant economic losses in many geographical areas, since within the colony, they spread quickly by contact between bees, as well as through the drift of infested bees spreading from one colony to another (Bailey, 1983). The tracheal mite *A. woodi* has invaded Asia, Europe, parts of Africa, North and South America except Australia, New Zealand, Norway and Sweden, due to its wide distribution, it is thought to be related to the loss of honeybee colonies in India (Singh *et al*., 2013; Shakib and Mehdi, 2016).

In Latin America, Maggi *et al*. (2016) identified its presence when checking the health status of bees in different countries and reported frequencies of 1 to 2.7% in Uruguay and 2.7% in the state of Táchira in Venezuela. European honeybee colonies in Europe, Asia and North America suffer large population losses, compared to bees in other parts of the world that manage to successfully survive pathogens (Coelho *et al*., 2015). In Mexico, *A. woodi* caused serious economic losses for beekeepers during the first years after its detection (Wilson and Nunamaker, 1983).

However, the humid tropic region of Mexico has been little studied, therefore, diseases being the probable causes of CCD, the objective of this work was to determine the frequency and levels of infestation of *V. destructor* and *A. woodi,* as well as the frequency and levels of infection of *Nosema* spp. in Tabasco, Mexico.

Materials and methods

Geographical location of apiaries

The state of Tabasco has a territorial extension of 2 446 100 ha. It borders to the north with the Gulf of Mexico and Campeche; to the east with Campeche and the Republic of Guatemala; to the south with Chiapas; to the west with Veracruz de Ignacio de la Llave, in Tabasco the existing climates are hot humid with rains all year round Af, hot humid with abundant rains in summer Am and hot subhumid with rains in summer A(w) (INEGI, 2017). Tabasco is divided into five physiogeographic subregions.

1) Centro Subregion, it is located within the hydrographic region of the Grijalva River, to which the Chontalpa Subregion and Sierra Subregion also belong. Its area is 2 572.84 km², which represents 10.51% of the total of the state. This subregion is formed by mostly low terrains and some swampy areas mainly, in the municipalities of Centro and Nacajuca. As can be seen in Figure 1, it has vegetation of grasslands, *tulares* and rainfed agriculture, with cultivations of corn, papaya, banana and cacao standing out.

2) Chontalpa Subregion, it is in the westernmost part of the state, bordering the states of Veracruz and Chiapas, this subregion is located within the hydrographic region of the Grijalva River, to which the Centro and Sierra Subregions also belong. It has an area of 7 606.09 km², which represents 31.08% of the total of the state. The terrain is mostly flat with few regular elevations of little importance. As can be seen in Figure 1, this subregion has vegetation of grasslands, *tulares*, rainfed agriculture, secondary vegetation, medium rainforest and mangroves, the plant associations

of the savannah are next to the rainforest and are basic for livestock farming. There are commercial plantations of citrus, cacao and various forest species such as eucalyptus. This subregion is the main producer of cacao, sugarcane, pineapple, lemon and orange, also has two of the three sugar mills existing in the state.

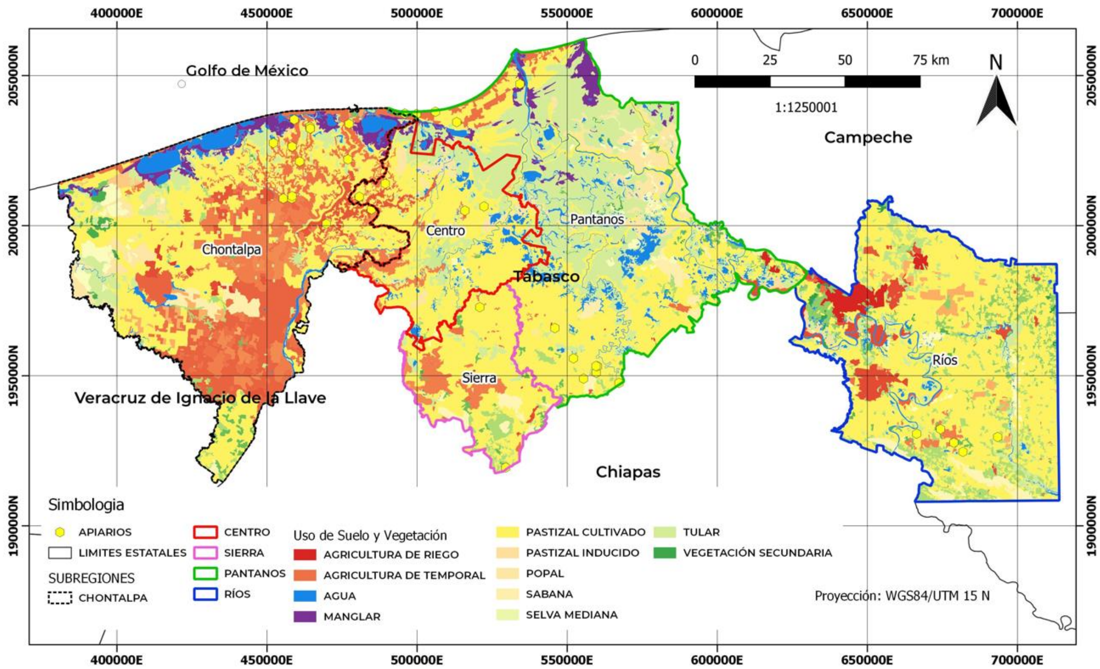

Figure 1. Geolocation of apiaries, subregions, land use and vegetation of Tabasco, Mexico.

3) Pantanos Subregion, it is in the central-northwest part of the state and as its name indicates, this area is where the Grijalva and Usumacinta rivers meet, forming numerous marshes, lagoons and swamps when the water stagnates due to the flat relief and with little or no variation. This subregion is located within the hydrographic region of the Usumacinta River, to which the Ríos Subregion also belongs. Its area is 6 588.39 km², which represents 33.74% of the total of the state. As can be seen in Figure 1, this subregion has vegetation of grasslands, *tulares*, rainfed agriculture, secondary vegetation, medium rainforest and mangroves. In this subregion is the biosphere reserve of the Pantanos de Centla, a natural heritage of humanity; which protects and conserves an extension of 302.706 hectares of wetlands, one of the largest areas of Mesoamerica with this ecosystem. This subregion is the one that preserves the highest concentration of rainforest reserve on the borders with the Republic of Guatemala.

4) Ríos Subregion, it is in the easternmost part of the state, on the borders with the state of Campeche and the Republic of Guatemala. It is named for the large number of rivers that cross it, including the Usumacinta River, the mightiest in the country, and the San Pedro Mártir River. This subregion is located within the hydrographic region of the Usumacinta River, to which the Pantanos Subregion also belongs. It has an area of 6 234.2 km², which represents 24.67% of the total of the state, the relief is flat with some hills and some mountainous areas that do not exceed 600 masl. As can be seen in Figure 1, this subregion has vegetation of grasslands, *tulares*, rainfed agriculture, secondary vegetation and medium rainforest.

5) Sierra subregion, this subregion is located within the hydrographic region of the Grijalva River, to which the Chontalpa and Centro subregions also belong. It has an area of 1 799.38 km², which represents 7.35% of the total of the state. The geography agrees more with that of the Sierra Madre of Chiapas than with that of the rest of Tabasco because, while the Tabasco relief is characterized by being flat and regular, this area has a large concentration of elevations, none of them greater than 1 000 masl, the climate also varies in this subregion, with some of the highest annual rainfall in the country occurring in the mountains (INEGI, 2017).

Obtaining the sample

The present work was carried out during October to November 2020, in apiaries of cooperating beekeepers from the five subregions, whose geolocation, land use and vegetation can be seen in Figure 1. From inside each brood chamber, a sample of 200-300 adult worker bees was taken in a 250 ml plastic bottle that contained 100 ml of 96% ethanol, making a total of 112 colonies. The samples were transferred to the biotechnology laboratory of the National Technological Institute of Mexico, Zona Olmeca campus (latitude 15Q 515233; longitude 15Q 2005221; 3 masl) for their conservation in refrigeration at 4 ºC until their analysis.

Laboratory work

Determination of the infestation frequencies of *V. destructor* and *A. woodi* and frequency of infection of *Nosema* spp.

For the determination of the frequency of infestation of each of the pathologies, the total of samples N= 112 colonies was considered. The frequencies [Frequency of *V. destructor* in capped brood (FVCO), frequency of *V. destructor* in adult bees (FVAA), frequency of *Nosema* spp. (FN) and frequency of *A. woodi* (FAW)] were obtained by the following formula: frequency= (number of positive colonies/112 total colonies) ∗ 100 (Thrusfield, 2007).

Determination of the level of infestation of *V. destructor*

In the present study, the level of infestation of *V. destructor* in capped brood (NIVCO) and the level of infestation of *V. destructor* in adult bees (NIVAA) were evaluated.

Determination of the level of infestation of *V. destructor* in capped brood (NIVCO)

To evaluate the NIVCO, a honeycomb fragment with capped brood (10 x 15 cm) was taken in each of the 112 colonies, from which the operculum of 100 cells was removed, to extract the larvae and quantify the number of total *V. destructor* mites. The inside of each cell was examined with the help of an otoscope. The NIVCO is the result of the number of cells infested with *V. destructor* mites divided by 100 cells analyzed (De Jong *et al*., 1982).

Determination of the level of infestation of *V. destructor* in adult bees (NIVAA)

According to the methodology proposed by De Jong *et al*. (1982), by stirring the samples of adult bees of each colony in 96% alcohol, the mites attached to the body of these bees were detached and then the alcohol was poured into a container with a mesh, which allows the passage of the mites through it. The NIVAA was determined by dividing the total number of *V. destructor* recovered by the total number of bees multiplied by 100.

Determination of the level of infection of *Nosema* spp. (NIN)

From each sample per colony, in a porcelain mortar, 20 bee abdomens were macerated in 10 ml of sterile distilled water (0.5 ml of sterile distilled water per abdomen). According to Cantwell (1970), an aliquot of the homogenate was deposited in a hemocytometer for the quantification of spores using an optical microscope (Leica brand, model DM 500, with integrated digital camera). The formula to determine the infection levels used was: number of spores per bee= volume of distilled water * number of spores per quadrant/volume per field. For the determination of the NIN, the parameters proposed by Jaycox (1960) were taken as a reference.

Determination of the level of infestation of *A. woodi* (NIAW)

From each sample per colony, 25 adult bees were taken at random according to OIE (2008), each bee had its head detached along with the first pair of legs, leaving the thoracic ring visible, then the tracheas were taken, making preparations for their observation under the stereoscopic microscope (Zeizz® brand, Stemi model, with integrated digital camera Mod. Axiocam 105).

Statistical analysis

The data obtained were subjected to a descriptive statistical analysis, tests of variance verification, detection of outliers and Kruscal-Wallis (H) test for the comparison of the medians. The simple linear correlation between infestation indices (r) was calculated. The data in percentage were transformed using the arcsine equation (Steel and Torrie, 1988). The programs Stat Graphics Centurion® and Minitap version IX® were used.

Results and discussion

Frequency and levels of infestation of *V. destructor* in capped brood

At the state level, an FVCO= 69.64% and a NIVCO= 13.86 ±17.96% were found. The highest infestation was found in the Chontalpa subregion 18.33 ±23.45, followed by the Pantanos (11.96 ±13.66) and Centro (10.5 ±11.49) subregions, finding values below the permitted limit (10%) in the Ríos (9.5 ±9.67) and Sierra (5.5 ±4.65) subregions, these results are possibly due to the nectar flow period, which varies between the different subregions.

As can be seen in Figure 2A, there is a difference of more than 3 to 1 between the smallest and largest standard deviation, which indicates that the data do not conform to a normal distribution. The Kruskal-Wallis test (H= 3.91; p= 0.418) indicates no significant differences between subregions at 95% confidence. However, these differences, although not statistically significant, are possibly due to the fact that the nectar flow varies in the different subregions.

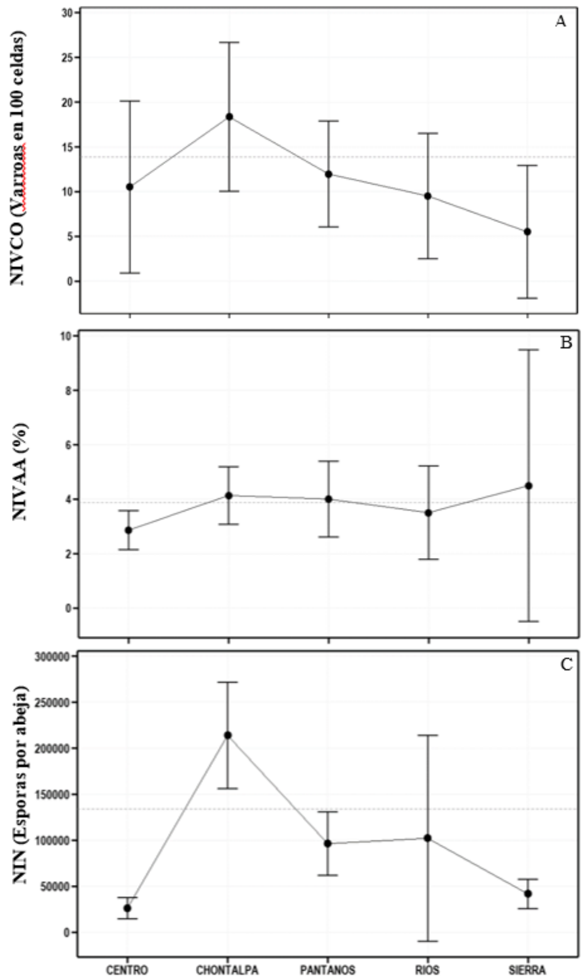

Figure 2. Infestation of *Varroa* in capped brood (NIVCO), infestation of *Varroa* in adult bees (NIVAA) and infestation of *Nosema* (NIN) in the subregions of Tabasco, Mexico.

When using the municipalities as a source of variation, the maximum level of NIVCO= 40.33 ±55 ±16% was found in the municipality of Cárdenas. The lowest level was found in the municipality of Jalapa (5.5 ±6.36) and Tacotalpa (5.5 ±4.95) and Centla (5.57 ±4.72). The great heterogeneity within the municipalities of the same subregion is observed. Of 78 (100%) positive diagnoses, 44 (56.41%) resulted in ≤ 9; 11 (14.1%) resulted in 10 to 15 and 23 (29.49%) resulted in 16 to 104 varroas in 100 cells. There is a relatively strong correlation between NIVCO and NIVAA (N= 112; r= 0.58; *p*= 0.0001).

Frequency and levels of infestation of *V. destructor* in adult bees

At the state level, an FVAA= 92.86% was found, with a NIVAA= 3.88 ±3.33%. The levels of infestation observed in the different subregions were as follows: Sierra (4.5 ±4.75), Chontalpa (4.14 ±3.35%) and Pantanos (4 ±3.79%), Ríos (3.51 ±2.97%) and Centro (2.87 ±1.13%). As can be seen in Figure 2B, there is a difference of more than 3 to 1 between the smallest and largest standard deviation, which indicates that the data do not conform to a normal distribution. The Kruskal-Wallis test (H= 0.77; *p*= 0.94) indicates that there are no statistically significant differences between subregions at 95% confidence.

On average, all subregions are below 5%, which is taken as a reference as the maximum permitted level that the colony must have based on DOF (1994). However, the coefficients of variation by subregion express a high variability between the colonies of the same apiary. When using municipalities as a source of variation, it was found in the municipality of Cárdenas (NIVAA= 10.02 ±4.98%) and the municipality of Jalapa (NIVAA= 6.13 ±6.9%), being the only municipalities that exceed the permissible limit according to DOF (1994). Lower levels were found in the municipality of Paraíso NIVAA= 2.55 ±1.1%, Centla NIVAA= 2.67 ±1.83%, Centro NIVAA= 2.85 ±1.09% and Jalpa NIVAA= 2.88 ±1.27%. As can be seen in Figure 2B, there is a wide variability between the values of the colonies, which is possibly due to the effect of the subregions.

In the present investigation, a high frequency in *V. destructor* was found. However, colonies that exceed the permissible limits were detected, according to DOF (1994). Similar works carried out by Martínez *et al*. (2011), in a comparative study between managed colonies and wild swarms, conducted in Mérida, Yucatán, reported FIVAA= 62.9%; NIVAA= 1.7 ±0.26 (mites/100 bees) in managed colonies; FIVAA= 55.1%; NIVAA= 1.96 ±0.44 (mites/100 bees) in wild swarms (non-significant differences). According to Vaziritabar *et al*. (2016); Masaquiza *et al*. (2019), certain bee populations have tolerance to the mite *V. destructor*, as their defense mechanisms allow them to maintain infestation rates in permissible ranges.

Frequency and levels of infection of *Nosema* spp

At the state level, an FN= 91.96% was found, with a NIN= 133 738 ±156 221 spores/bee, which corresponds to a very light intensity of infection. A frequency of 80 to 100% was found in the five subregions; the frequencies observed were as follows: Sierra (FN= 100%), Chontalpa (FN= 95.35%), Pantanos (FN= 94.44%); Centro (FN= 83.33%) and Ríos (FN= 80%). Although in all subregions they correspond to a very light intensity of infection according to Jacox's (1960) scale, on average the highest infection was found in Chontalpa (NIN= 213 720 ±182 724 spores per bee), followed by Ríos (NIN= 102 083 ±176 039 spores per bee), Pantanos (NIN= 96 324 ±98 595 spores per bee), Sierra (NIN= 41 667 ±15 138 spores per bee) and Centro (NIN= 26 250 ±16 084 spores per bee).

As can be seen in Figure 2C, there is a difference of more than 3 to 1 between the smallest and largest standard deviation, which indicates that the data do not conform to a normal distribution. The Kruskal-Wallis test (H= 0.79; p= 0.944) indicates that there are no statistically significant differences between the subregions at 95% confidence. When using the municipalities as a source of variation, the maximum NIN was found in the municipalities of Cunduacán (NIN= 250 000 ±220 440) and Comalcalco (NIN= 240 948), followed by Macuspana (NIN= 116 447 ±109 612) and Tenosique (NIN= 102 083). The remaining 63.64% of the municipalities had NIN≤ 90 000 spores per bee. Similar studies conducted in Yucatán reported the presence of *Nosema* spp. in 74.0% of managed colonies and an infection level of 1 430 x 10^3 ±232 x10^3 (spores/bee); in wild swarms, a frequency of 53% was observed, with an infection level of 1 416 x 10^3 ±264 x10^3 (spores/bee).

The results observed in the present study coincide with what was reported by Martínez and Catzín (2012), who reported a high frequency of *Nosema* spp. (99%) in the samples collected, concluding that the presence of *Nosema* spp. has been increasing over the years in Campeche. In the present work, significant differences were found between apiaries (very light infestation level), considering that the samples were collected during the same season of the year, these results could suggest some effect due to the management those beekeepers carry out in the colonies.

The lower values found in wild swarms could be explained according to some authors such as Bailey (1983), who reported that the constant manipulation of colonies raises stress levels, causing a higher incidence of diseases. The spores of *Nosema* spp. are resistant to the environment, being found in the waste of bees or honey, in addition to remaining viable for a year. The number of spores of *Nosema* spp. increases with increasing levels of infestation of *V. destructor*, due to the reduction of hemolymph in infested bees, favoring the multiplication of spores (Fries *et al*., 2013); however, our results showed that there is no significant correlation between these variables (r= -0.05; *p*= 5.5612).

According to Medina *et al*. (2014), they collected bee samples in 25 commercial apiaries, distributed in 15 municipalities belonging to three ecological zones in the state of Zacatecas; (n= 151 colonies) and in mid-spring 2011 (n= 148 colonies), finding 4.7% of the population analyzed in spring positive for *Nosema* spp.; while most cases of *Nosema* spp. (86%) were detected in the semi-dry semi-warm zone, evidencing the effect of the season of the year; they also indicated that *V. destructor* is the most common parasitosis of adult honeybees in Zacatecas.

On the other hand, Bravo *et al*. (2014) found 16% of samples positive for *Nosema* spp. in five municipalities of the state of Oaxaca. Nava (1996), in the southern zone of Jalisco in 1996, found a very light infection in 90% of the colonies evaluated, years later, Tapia *et al.* (2017) analyzed the relationship of environmental factors with FN in eight municipalities in the south-southeast of the state of Jalisco (two municipalities with temperate subhumid climate, six with hot subhumid climate), in this study they found that 100% of the samples were positive, 83.75% had between 1 to 5 million spores/bee (light infection), 13.3% presented 0.01 to 1 million spores/bee (very light infection), 2.6% had 5 to 10 million spores/bee (regular infection) and 0.25% presented 10 to 20 million spores/bee (semi-severe infection).

These authors reported that low negative correlations were found (r= -0.35) (*p*= 0.01) between altitude and the intensity of infection of *Nosema* spp.; likewise, they found a weak negative correlation between the intensity of infection of *Nosema* spp. (r= -0.12) with the municipalities that have more annual rainfall than the average (953 mm), this relationship was not significant (*p*= 0.16); however, the ambient temperature showed a medium correlation with the intensity of infection of *Nosema* spp. (r= 0.44; *p*= 0.01). This suggests that as the temperature increases above the average of 21°C in the municipalities evaluated, the intensity or severity of the infection will increase, based on these results, Tapia *et al*. (2017) have suggested the possibility of doing biological control, removing the oldest honeycombs in the colonies each year, and carrying out the annual change of queens.

Presence of *V. destructor* and *Nosema* spp.

The presence of *V.* destructor-Nosema spp. causes a high mortality of bees, even though the number of spores of *Nosema* spp. in the organism of these insects is much lower than in cases of infection of this microsporidium alone (Hinojosa and González, 2004). As can be seen in Figure 3, 63.40% of triple coinfections (NIVCO-NIVAA-NIN) and 23.21% of double co-infections (NIVAA-NIN) were found, with the rest of the possible combinations being less frequent.

According to Bravo *et al*. (2014), by microscopic analysis and confirmation by molecular methods, they reported the frequency of *N. ceranae* in the V region of Valparaíso, Chile, confirming the absence of *N. apis*. This shows that molecular techniques are of great importance to complement conventional microscopy techniques, in epidemiological studies of *Nosema* spp., which should be considered in human resources training programs and policies for the monitoring of population dynamics and appropriate control of these pathogens in the face of the latent risk of increasing the infection of the colonies in Mexico.

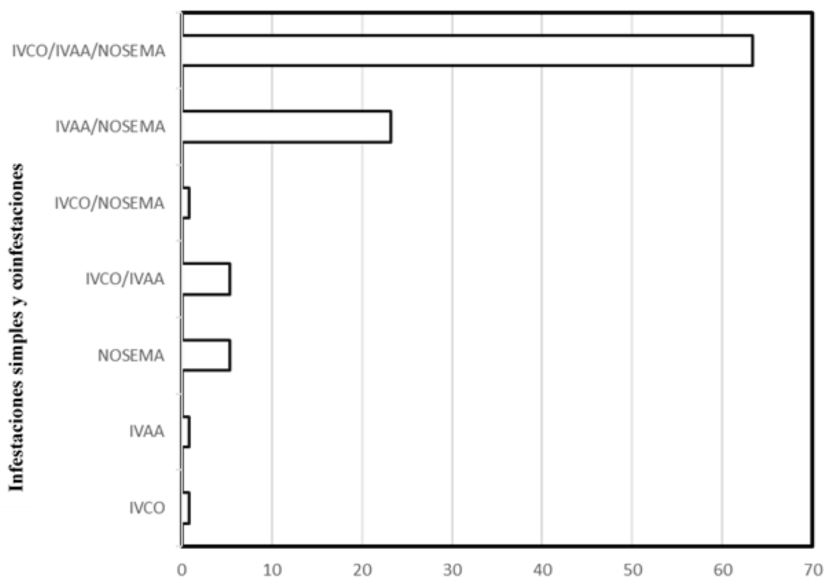

Figure 3. Simple, mixed and triple infestation of *Varroa* in capped brood (IVCO), infestation of *Varroa* in adult bees (IVAA) and infestation of *Nosema* (IN) in Tabasco, Mexico.

Frequency and levels of infestation of *A. woodi*

In the present investigation, the presence of tracheal mites was not detected in any of the samples analyzed. García and Arechavaleta (2018), in a study conducted in the state of Morelos, Mexico, reported a prevalence of *A. woodi* of 0.02 and that 10.3% of the apiaries included in the study had at least one colony positive for this disease. The average infestation percentage in positive colonies was 7.32 ±0.75, with a minimum infestation level of 5% and a maximum level of 20%. On the other hand, Martínez *et al*. (2015), in a study conducted in commercial colonies in the state of Yucatán, Mexico, found no presence of *A. woodi*.

Conclusions

The presence of *V. destructor* was observed in 92.86% of the colonies at the state level. However, it was observed that there are differences in the frequencies and levels of infestation between the different subregions of the state of Tabasco. It is important that beekeepers establish treatment schedules for the control of *V. destructor* according to the conditions of each subregion.

The presence of *Nosema* spp. in the state of Tabasco was confirmed, since, although beekeeping is an important activity for the state, there are no studies that have confirmed its presence. A frequency of 91.96% was observed, with an infection level of 133 738 ±156 221 spores/bee.

The presence of *A. woodi* was not observed in the state, so possibly the frequency of this parasite is low.

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