

# Infestation levels of *Varroa destructor* and *Nosema* spp. in africanized bee (*Apis mellifera*) colonies during the dry season in the semiarid region of Piauí state

*Nível de infestação por Varroa destructor e ocorrência de Nosema spp. em colônias de abelhas africanizadas (Apis mellifera) durante o período seco em uma região semiárida do estado do Piauí*

Jossandra de Jesus Silva do Nascimento<sup>1</sup> , Maria Beatriz Silva Ferreira<sup>1</sup> ,  
Catiana da Conceição Vieira Melquíades<sup>2</sup> , Juliana do Nascimento Bendini<sup>\*</sup> 

**ABSTRACT:** This study aimed to assess infestation levels of the mite *Varroa destructor* and microspore *Nosema* spp. in apiaries in the Picos microregion of Piauí state, Brazil, during the dry season. To that end, bees were collected (n=31) from 19 apiaries between September and October 2016. Infestation levels of the parasites were analyzed at the Laboratory of the Bee Technology Center, an institution affiliated with the Senador Helvídio Nunes de Barros Campus of the Federal University of Piauí. *Varroa destructor* was identified in 54% of the colonies, with infestation levels varying from 0 to 10.54%, and an average of 3.31% ± 4.12. Analysis of *Nosema* spp. revealed that all the colonies sampled exhibited microsporidia, albeit at low (1 to 1.4 spores/bee) and very low levels (0.35 to 0.95 spores/bee) of infestation. Given the relevance of beekeeping in the semiarid region of Piauí, it was concluded that although infestation levels were deemed low, there is a need to regularly monitor the health of colonies in the apiaries.

**KEYWORDS:** Beekeeping; Honey production; Bee health.

**RESUMO:** Objetivou-se avaliar o nível de infestação do ácaro *Varroa destructor* e do microsporídio *Nosema* spp em apiários da microrregião de Picos, Piauí, durante o período seco. Foram realizadas coletas de amostras de abelhas (n=31) em 19 apiários, entre setembro e outubro de 2016. As análises foram realizadas no Laboratório do Centro de Tecnologia Apícola. Observou-se a ocorrência de *Varroa destructor* em 54% das colônias, sendo que os níveis de infestação variaram de 0 a 10,54%, com média de 3,31% ± 4,12. A pesquisa referente à *Nosema* spp revelou que todas as colônias amostradas apresentavam o microsporídio, no entanto em níveis baixos (1 a 1,4 esporos/abelha) e muito baixos (0,35 a 0,95 esporos/abelha) de infestação. Considerando a relevância da apicultura para a região semiárida do Piauí, concluiu-se que embora os níveis de infestação tenham sido considerados baixos, ressalta-se a necessidade de monitoramentos periódicos quanto à sanidade das colônias nos apiários.

**PALAVRAS-CHAVE:** Apicultura; Caatinga; Nordeste; Produção de mel; Sanidade Apícola.

## INTRODUCTION

Different biotic and abiotic factors pose a threat to bee health and, indirectly, the maintenance of biodiversity and global food production. Research suggests that losses of bee colonies are frequently associated with interactions between pathogens and other stress factors, including parasites (NAZZI et al., 2012). Among these organisms, Tesovnik et al. (2017) highlight the mite *Varroa destructor* as a possible co-factor in the decline of global bee populations.

When infesting honeybees, the mite can cause severe losses in bee colonies (*Apis mellifera*), compromising yield and killing both young and adult bees (WILSON-RICH et al., 2008). According to Tesovnik et al. (2017), it is one of the most common mites in bee colonies and a vector of viruses that cause diseases such as sacbrood, paralysis and wing deformities (KUSTER; BONCRISTIANI; RUEPPELL, 2014). Additionally, Bermejo and García Fernández (1997) reported

<sup>1</sup>Grupo de Estudos sobre Abelhas do Semiárido Piauiense, Universidade Federal do Piauí, Picos, Brasil

<sup>2</sup>Programa de Pós-graduação em Ciência Animal, Universidade Federal do Vale do São Francisco, Petrolina, Brasil.

\*Corresponding author: jbindini@ufpi.edu.br

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that the stress caused by *Varroa destructor* parasitism in bees may be linked to the occurrence of nosemosis, a severe and prevalent bee disease caused by microsporidia (*Nosema apis* and *Nosema ceranae*), intracellular parasites that infect the midgut epithelial cells of the adult bee hosts *Apis mellifera* and *Apis cerana*, respectively (SINPOO et al., 2018).

Bees are infected by *Nosema* spp. through the ingestion of mature spores, possibly during cleaning activities, contaminated pollen while exchanging food with other bees (trophallaxis) (FORSGREN; FRIES, 2010) or contaminated water (CHEN et al., 2009).

Beekeeping in Brazil is marked by the introduction of the African bee (*Apis mellifera scutellata*) and crosses between this subspecies and other European breeds introduced on the American continent. These crosses resulted in the Africanized bee (SOUZA; GRAMACHO; CASTAGNINO, 2012), currently used by beekeepers across the country and, according to Message et al. (2012), considered more resistant to different pathogens and parasites than European bees. However, in Brazil, epidemiological surveys are scarce (MESSAGE; TEIXEIRA; DE JONG, 2012), with most studies focusing on the South and Southeast of the country (CARNEIRO et al., 2007, PINTO et al., 2011, PINTO et al., 2012, CARNEIRO et al., 2014, OCTAVIANO-SALVADÉ et al., 2017, SCHAFASCHEK et al., 2019).

The dry season in the Brazilian semiarid is characterized by high temperatures, with low rainfall and relative humidity (MELQUÍADES; BENDINI; MOURA, 2020). These factors, combined with the scarcity of floral resources (BENDINI et al., 2021), favor the weakening of bee colonies, which may become more susceptible to pathogen infestation (COTTER et al., 2011). According to the authors, the immune response of these insects is affected by the low availability of essential amino acids and resulting peptide synthesis, with the lack of carbohydrates reducing the availability of the metabolic energy needed for immune system processes.

In Piauí state, beekeeping has provided employment opportunities and income for family-run small and medium-scale beekeeping farms, especially in the semiarid region (VELOSO FILHO et al., 2012). Given that bee diseases can contribute to weakening of the colony, hive abandonment and yield losses in apiaries in the region, monitoring the health of bees in apiaries in semiarid Piauí is important and recommended. In this respect, the present study aimed to assess infestation levels of the mite *Varroa destructor* and microspore *Nosema* spp. in apiaries in the Picos microregion of Piauí state during the dry season.

## MATERIAL AND METHODS

Bees were collected from apiaries in the Picos microregion of Piauí, during the dry season (September to October 2016). To that end, prior contact was made with representatives of associations and/or cooperatives in the municipalities of Itainópolis, Jaicós and Massapê in Piauí to provide guidance

on the damage caused by the pathogens (*Varroa destructor* and *Nosema* spp.) and the importance of monitoring the health of their colonies.

A total of 19 apiaries were sampled in Massapê do Piauí (7° 27' 43" S, 41° 7' 18" W), Jaicós (7° 21' 29" S, 41° 7' 60" W) and Itainópolis (7° 27' 11" S, 41° 28' 10" W). *Varroa destructor* infestation was investigated using the methodology described by Stort et al. (1981), modified by Message (1983). Between 100 and 200 adult bees were collected directly from the center frame of 10% of the apiaries visited. The samples (n=31) were stored in sealed transparent jars containing 200 ml of 70% alcohol, labeled with information on the collection location, name of the beekeeper and apiary number.

At the Laboratory of the Bee Technology Center (CENTAPI), an institution affiliated with the Senador Helvécio Nunes de Barros Campus of the Federal University of Piauí in Picos, each sample was agitated to remove the mites from the bees with the aid of a sieve attached to a PET plastic bottle, so that the bees were retained and the mites fell into a white plastic wash basin to facilitate visualization. Next, the mites and bees were counted separately and the infestation level in each hive was calculated using the following formula: *Varroa* infestation rate (%) = (no. of *Varroa* mites / no. of bees) X 100.

The same colonies were used to investigate the occurrence of *Nosema* spp., with the hive entrances sealed using a foam strip. This made it possible to obtain only field bees, frequent hosts of *Nosema* spp., which were stored in flasks containing 70% alcohol, totaling 31 samples of at least 60 adult bees per flask.

In the laboratory, the abdomens of the bees were macerated, sieved, filtered and diluted in distilled water to count the spores under an optical microscope (400x) using a Neubauer chamber. The following formula was used: No. of spores per bee = Total no. of spores / 80) \* 4000000 (VALADARES et al., 2016).

The protocol for laboratory diagnostic techniques for bee diseases and pests was used as reference (VALADARES et al., 2016) (Table 1).

## RESULTS AND DISCUSSION

The results of *Varroa destructor* and *Nosema* spp. infestation analysis (Table 2) revealed that the mite was detected in 54% of the colonies and all the apiaries studied were infected by *Nosema* spp. The levels of infestation did not pose a risk to

**Table 1.** Level of *Nosema* spp. infestation in honeybees.

Infestation Level	No. of spores per bee (millions)
Null	< 0.01
Very low	0.01 – 1.00
Low	1.01 – 5.00
Moderate	5.01 – 10.00
Semi-severe	10.01 – 20.00
Severe	>20.00

**Table 2: *Varroa destructor* and *Nosema* spp. infestation levels in African bee colonies in semiarid Piauí.**

Sample	Municipality	<i>Varroa</i> Infestation Rate (%)	Number of <i>Nosema</i> spp. spores (millions/bee)	<i>Nosema</i> spp. Infestation Level
1	Itainópolis	1.35	800000	Very low
2	Itainópolis	0.91	750000	Very low
3	Itainópolis	2.5	900000	Very low
4	Itainópolis	1.1	850000	Very low
5	Itainópolis	1.9	850000	Very low
6	Itainópolis	4.3	900000	Very low
7	Itainópolis	0	400000	Very low
8	Itainópolis	5.4	1000000	Very low
9	Itainópolis	0	750000	Very low
10	Itainópolis	0	750000	Very low
11	Jaicós	0	500000	Very low
12	Jaicós	0.92	800000	Very low
13	Jaicós	0.46	600000	Very low
14	Jaicós	0.46	600000	Very low
15	Massapê do Piauí	0	500000	Very low
16	Massapê do Piauí	0	600000	Very low
17	Massapê do Piauí	4.5	1250000	Low
18	Massapê do Piauí	0	600000	Very low
19	Massapê do Piauí	1.2	700000	Very low
20	Massapê do Piauí	6.1	950000	Very low
21	Massapê do Piauí	5.6	1000000	Very low
22	Massapê do Piauí	1.9	1400000	Low
23	Massapê do Piauí	11.9	1200000	Low
24	Massapê do Piauí	0	350000	Very low
25	Massapê do Piauí	0	400000	Very low
26	Massapê do Piauí	0	700000	Very low
27	Massapê do Piauí	0	350000	Very low
28	Massapê do Piauí	0	900000	Very low
29	Massapê do Piauí	0	850000	Very low
30	Massapê do Piauí	0	700000	Very low
31	Massapê do Piauí	10.6	1000000	Very low

the apiaries since no nosemosis symptoms were observed in the colonies sampled.

### *Varroa destructor* infestation level

The average mite infestation level recorded in the region studied here ( $3.31\% \pm 4.12$ ) is consistent with other investigations carried out in semiarid regions of the states of Pernambuco and Rio Grande do Norte (CLEMENTINO; GALINDO; MILFONT, 2016; MOREIRA et al., 2017) and other regions of the country (PINTO et al., 2011; PINTO et al., 2012; PINTO et al., 2015; WIELEWSKI et al., 2013).

In 27 years of research on the occurrence of *Varroa* in Brazil, Castilhos et al. (2019) found no evidence of increased infestation in *Apis mellifera* colonies in the country. Other authors have reported that this pathogen is not a major problem for Brazilian beekeepers.

It is important to note that the hereditary hygienic behavior of *Apis mellifera* provides resistance to *Varroa destructor* (CHERUIYOT et al., 2018). Castagnino, Pinto and Carneiro (2016) also found that, compared to European honeybees (Italian, Caucasian and hybrid), their Africanized counterparts are more efficient at removing dead offspring and are therefore considered more hygienic. The authors emphasized

that the hygienic behavior of bees is one of their most efficient mechanisms in defending the colony from possible pathogens.

Evangelista et al. (2015) recorded a *Varroa destructor* infestation level of 0 to 12% in worker bees and 0 to 18% in offspring in apiaries from the municipality of Teresina in Piauí state, with an increasing trend in months when less food is available. Santos et al. (2011) highlighted the influence of floral resource availability on *Varroa* infestation levels in adult bees.

In regard to the results of the present study, the *Varroa destructor* infestation level was expected to be high during the dry season in the semiarid, largely because the conditions characteristic of this period (scarce floral resources for bees, high temperatures, and low relative humidity). However, 80.64% of the colonies sampled exhibited an infestation level of 0 to 5.

### Nosema spp. occurrence

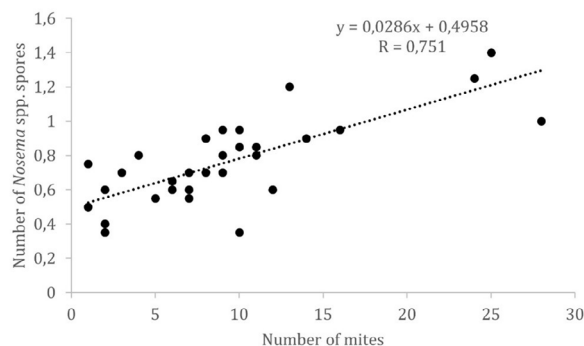
According to Teixeira et al. (2013), the use of molecular techniques to identify individual species from the genus *Nosema* is relatively recent. As such, species were not identified in this study or most of those surveyed.

Santos et al. (2011) investigated the presence of *Nosema* spp. in apiaries in different regions of São Paulo state and found that 95.7% of the colonies were infected, albeit at low levels. In Pernambuco state, Clementino et al. (2015) assessed *Nosema* spp. infestation and observed moderate to semi-severe levels.

It is important to underscore that *Nosema* spp. was found in the bees collected from all the hives analyzed and, despite the low infestation levels observed, its presence could significantly reduce colony size, honey production and brood rearing (BOTIAS et al., 2013).

Mariani et al. (2012) confirmed the existence of parasitic interactions between *Varroa destructor* and *Nosema* spp. According to Bermejo and García Fernández (1997), the occurrence of *Nosema apis* may be associated with stress caused by *Varroa destructor* parasitism in bees. However, Bahreini and Currie (2015) emphasized that a better understanding of interaction between *Varroa destructor* and *Nosema* spp. is vital in developing new management strategies to mitigate colony losses. In this respect, linear regression was performed between the number of *Varroa destructor* mites and number of *Nosema* spp. spores in the samples collected (Figure 1).

In semiarid Piauí, the correlation coefficient of simple linear regression ( $r = 0.75$ ) revealed a correlation between the



Correlation significant at 1%.

**Figure 1.** Linear regression between the number of *Varroa destructor* mites and number of *Nosema* spp. spores.

variables, demonstrating a trend of positive association between the number of *Varroa destructor* mites and number of *Nosema* spp. spores present in the bees and corroborating the findings of Bermejo and García Fernández (1997) in European bees.

However, Teixeira et al. (2013) emphasized that more information is needed regarding the factors involved in the transmission and survival of this parasite to help clarify its impact on Africanized bee colonies.

As reported in the literature and confirmed in the present study, infestation levels of *Varroa destructor* and *Nosema* spp. in apiaries in the semiarid are considered low and can be reduced via prophylactic sanitary measures adopted by beekeepers. The selection and multiplication of colonies with hygienic behavior is an efficient *Varroa* control measure (CASTAGNINO; PINTO; CARNEIRO, 2016). In this respect, regular inspections, acquiring healthy colonies from reliable sources, the availability of quality water in apiaries and individual drinking fountains in hives are measures that can reduce contamination rates of the disease.

### CONCLUSION

*Varroa destructor* and *Nosema* spp. were detected in Africanized bee colonies in the Picos microregion of Piauí state, Brazil. Although the infestation levels recorded were low, there is a need for regular monitoring of bee health in apiaries.

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