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ARTICLE

Infestation level and ecological association of *Nosema* spp. (Microsporida: Nosematidae) and *Varroa destructor* (Mesostigmata: Varroidae) in honeybee colonies in south-west Nigeria

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Resumen

Nivel de infestación y asociación ecológica de *Nosema* spp. (Microsporida: Nosematidae) y *Varroa destructor* (Mesostigmata: Varroidae) en colonias de abejas del suroeste de Nigeria

Se estudió el nivel de infestación y la asociación ecológica de *Nosema* spp. *y Varroa destructor* en abejas. La tasa de infestación (Mn±SD) de *V. destructor* osciló entre el 1,05 ± 0,64% y el 9,02 ± 4,98%. La prevalencia de *Nosema* spp., en el estado de Osun, osciló entre baja y semisevera, mientras que en el estado de Ogun variaron de moderados a severos. Sin embargo, en ambos estados se observó infestación grave de esporas. Se reveló correlación lineal negativa (r = -0,329) entre *V. destructor* y *Nosema* spp. en las colonias de abejas. En general, todas las colonias presentaban una infección de moderada a grave por esporas de *Nosema* spp. y el 93% de las colonias tenían *V. destructor*, lo que requiere medidas urgentes de mitigación.

Palabras clave: *Apis mellifera adansonii*; Tasa de infestación; Nivel de asociación parasitaria y Severidad; *Nosema* spp; *Varroa destructor*.

Abstract

The level of infestation and ecological association of *Nosema* spp. and *Varroa destructor* was studied in honeybees. The infestation rate (Mn±SD) of *V. destructor* ranged from 1.05 ± 0.64% to 9.02 ± 4.98%. The prevalence of *Nosema* spp. in Osun State was found to range from low to semi-severe, while in Ogun state, infestation leves varies from moderate to severe. However, in both states severe infestation of spores was observed. A linear negative correlation (r = -0.329) was revealed between the *V. destructor* and *Nosema* spp. in honeybees colonies. In general, all the colonies exhibited moderate to severe infection by *Nosema* spp. spores and 93% of the colonies had *V. destructor*, which necessitates urgent mitigation measures.

Key words: *Apis mellifera adansonii*; Infestation rate; Level of parasitic association and Severity; *Nosema* spp.; V*arroa destructor*.

Introduction

Honeybees are referred to as social insects with three castes, queen, drones, and worker bees. They are of economic value to agricultural outputs with respect to pollination services in the ecosystem. In general, they contribute to the survival and sustenance of mankind and animals. However, these insects may have environmental stressors leading to either a population decline or in their output. Different abiotic and biotic factors negatively impact health of bees and food production globally. Colony losses are associated with the interaction between honeybees, pathogens, pests, and other environmental stressors, either abiotic or biotic (Nazzi *et al*. 2012). *Varroa destructor* Anderson & Trueman, 2000 has been widely identified as one of the co-factors contributing to decline of honeybees population by infestation causing the death of honeybees, resulting in severe colony losses in most regions across the Globe (Cox-Foster *et al.* 2007; Wilson-Rich *et al.* 2008, Currie *et al*. 2010, Neumann & Carreck 2010, Tesovnik *et al*. 2017; Do Nascimento *et al*. 2022). Stress caused by *V. destructor* to honeybees may expose the colony to *Nosema apis* Zander, 1909 and *Nosema ceranae* Fries *et al*., 1996 linked to the occurrence of nosemosis, a severe and prevalent bee disease caused by their *Nosema* Nägeli, 1857 microsporidia, which are intracellular fungi parasites that infect the midgut epithelial cells of adult honeybees of *Apis cerana* Fabricius, 1793 and *Apis mellifera* L., 1758 (Sinpoo *et al*. 2018). The symptoms of the disease include intestinal disorders (diarrhea), distended abdomens, unhooked wings; the activities of bee foragers may be likely reduced drastically, egg-laying activities of the queen may decrease, and general behavior is distorted. Therefore, *Nosema* infection often led to a reduction in honey crops and the acceleration of queen supersedure depending on the level of infestation (Sanford 2003, FAO 2006, 2018). Transmission of *Nosema* spores is mostly by fecal-oral route as these are mostly found in honeybee feces which are directly or indirectly ingested by adult workers. Thereafter spores develop in the intestines of the bee affecting the gut functions. Once the spores are expelled outside with fecal material, they may be swallowed by other members of the colony that become infected automatically (Sanford 2003, Akinwande *et al*. 2013, FAO 2018). There is limited information on the surveillance of ecological association of *Varroa destructor* and *Nosema* spp. and the level of infestation of these parasites in most colonies in southwestern Nigeria, a knowledge much needed to determine the health status of colonies of honeybees in the region. This study focused on assessing the infestation and ecological association of both parasites.

Materials and methods

Study areas

The study areas involved colonies located in four federal states of Southwest Nigeria, namely Ogun (Odeda and Osiele), Oyo (Iresa Adu and Ogbomosho), Osun (Okinni and Ibokun) and Ondo (Akure and Ikare-Akoko) (Fig. 1).

Bee farmers' awareness and orientation programme

Bee farmers (apiculturists) managing the sampled colonies were educated about the aims and objectives of the study and the expected benefits of its likely outcome. They were enlightened on implications of the likely damage caused by parasites and pests of honeybees, specifically *V. destructor* and *Nosema* spp. spores), and the importance of monitoring the health status of their colonies.

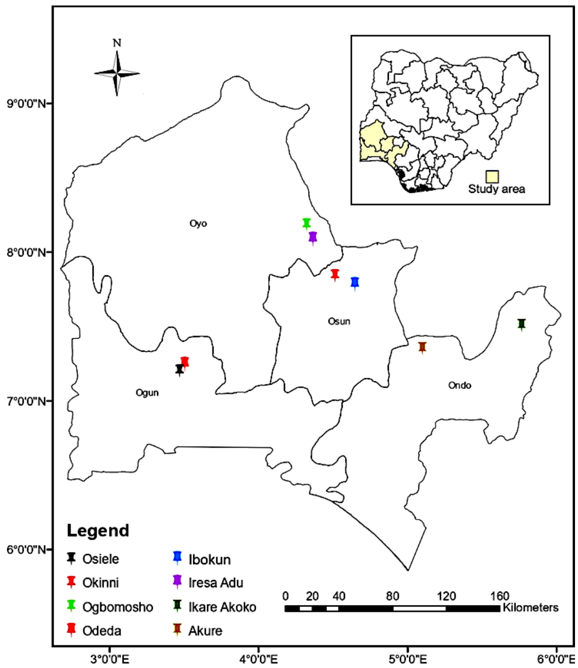

Figura 1. Mapa de áreas de estudio en el suroeste de Nigeria
Figure 1. Map of study areas within Southwest Nigeria.

Sample collection

Two honeybee colonies were selected for the study in each of the four Nigerian states. Colony sites were mostly used for agricultural activities. For each site verbal consent approval was obtained from the apiculturists, and one apiary was selected for the study. Prior to and during the study, none of the colonies selected across the four states received any parasitic treatment against either *V. destructor*, *Nosema* spp. and other parasites of honeybees. Three honeybees colonies per apiary per community were randomly selected for sampling. Global positioning (GPS) of each apiary was carried out and recorded accordingly for mapping.

Samples of honeybees (≥300) were collected directly from the center frame of each colony into glass jars (200 ml) containing 70% alcohol and taken to the laboratory for preservation and laboratory analysis within 72h. Samples were obtained monthly across the four states from June to December 2021.

Assessment of *Varroa destructor* infestation

Each ≥300 honeybee samples in 70% alcohol were agitated for 20s to dislodge the mites from the adult honeybees and separated with the aid of sieve (3-4 aperture) attached to a container jar so that the adult honeybees were retained, and the mites fell into a white plastic bowl (2L) to facilitate visualization. Thereafter, mites were counted, and the infestation level was calculated using the formula below (Dietemann *et al*. 2013):

$$\textit{Varroa infestation rate}(\%) = \frac{\textit{Number of Varroa mites}}{\textit{Number of honeybees}} \times 100$$

Assessment of *Nosema spp.* infestation

The abdomen of 100 honeybees per colony was macerated and homogenized into a 200 ml glass jar with 100 ml of distilled water. Thereafter, a drop of homogenized solution was taken and placed into Neubauer chamber (Hemocytometer) (Hawskley B.S. 748) to quantify the number of spores under an optical microscope (x400), as described by Fries *et al.* (2013). The following formula was used to determine the number of spores per honeybee (Valladares *et al*. 2016):

$$\textit{No. spores per honeybee} = \frac{\textit{Total number of spores}}{80} \times 4000000$$

The severity level of *Nosema* spp. spores' infestation in honeybees was categorized as either null, very low, moderate, semi-severe, or severe (Table 1) (Valladares *et al*. 2016). This process was repeated for other samples.

Data Analysis

IBM SPSS Statistics 29.0.2.0 version was used to get the mean, standard deviation and linear regression coefficient, p-value and R^2 using data obtained to determine the relationship between *Nosema* spp. spores and the *V. destructor*.

Results

The infestation levels of *V. destructor* and *Nosema* spp. vary across the apiaries studied in Southwest Nigeria (Table 2). *Varroa destructor* was detected in 93% of the colonies studied. The infestation rate (Mn±SD) of *V. destructor* ranged from 1.05 ± 0.64% to 9.02±4.98% across the four states studied, the average infestation rate across southwestern Nigeria was 3.26±4.42%. The number of *Nosema* spp. spores (Mn±SD) (millions/bee) ranged between 6.91±3.69 to 55.22±10.09 (millions/bee) in honeybee' colonies across the four selected states, the average value was 29.49±21.00 (millions/bee). Out of the 15 colonies sampled, 6.7% of the colonies exhibited low level of *Nosema* spp. spore' infestation. Within states, the infestation level was low to semi-severe in Osun, moderate to severe in Ogun and severe in Oyo and Ondo.

Infestation Level	No. 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

Tabla 1. Nivel de *Nosema* spp. infestación de abejas.

Table1. Infestation level of *Nosema* spp. in honeybees.

State	*Varroa destructor* Infestation	No. *Nosema* spores (millions/bee)	*Nosema* spp. Infestation Lv
Osun	1.05 ± 0.64	6.91±3.69	Low-Semi-severe
Ogun	9.02 ± 4.98	17.28±10.88	Moderate-Severe
Oyo	1.44 ± 1.50	44.95±1.28	Severe
Ondo	0.33 ± 0.09	55.22±10.09	Severe
	3.26±4.42	**29.49±21.00**	**Moderate-Severe**

Tabla 2. *Varroa destructor* y *Nosema* spp. Niveles de infestación en el suroeste de Nigeria. Mn±DE.

Table 2. *Varroa destructor* and *Nosema* spp. infestation levels in Southwest Nigeria. Mn±SD.

With regard to the association between *V. destructor* and *Nosema* infestations, we found that all the sampled colonies had both parasites except for colony J (Iresa Adu), which had only *Nosema* spores. The correlation coefficient shows a negative linear correlation between the variables (r = - 0.329), but the association is too weak (R^2= 0.1083; p=0.2311) between V. destructor and *Nosema* spp. (Fig. 2).

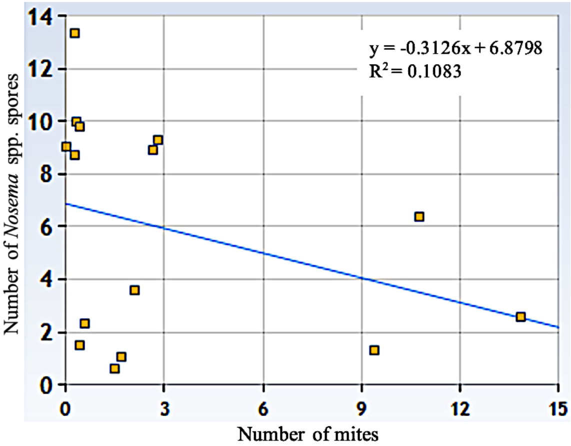

Figura 2. Regresión lineal que representa la relación entre *Varroa destructor* y *Nosema* spp.

Figure 2. Linear regression depicting the relationship between the *Varroa destructor* and *Nosema* spp.

Discussion

Beekeepers in many countries noticed a significant increase in the percentages of diseases that affect their honeybee colonies. Previous studies suggested that recent colony losses observed in Europe, the United States, Africa, and Nigeria may be due to synergist effects between *Nosema spp.* and *V. destructor* (Anderson & Trueman 2000, Higes *et al.* 2009; Gisder *et al.* 2010, Chemurot *et al*. 2017), but those observations are not fully substantiated. *Nosema* spp. was found in the guts of honeybees sampled across all the locations selected for the study. The infestation varies from low to severe. Osun and Ogun states had between low and severe infestation of *Nosema* spp. spores while Oyo and Ondo states experienced severe infestation. Botías *et al*. (2013) reported that the presence of *Nosema* spores at a severe level significantly reduces bees' population in the colony, honey production, and brood rearing. This implies that Oyo and Ondo states with severe infestation level of *Nosema* spp. spores may likely experience colony loss over time, hence the beekeepers in those states need to be cautious and counseled to apply control and mitigation measures. Mariani *et al*. (2012) confirmed the existence of parasitic interactions between *V. destructor* and *Nosema* spp. as observed in this study which need for caution. According to Bermejo & Fernández (1997), the occurrence of *N. apis* may be associated with stress caused by *V. destructor* parasitism in honeybees. However, it was observed that all the apiaries had *Nosema* spp. spores while 93% of the apiaries had *V. destructor*. It was observed that there was ecological association between *V. destructor* and *Nosema* spp. in most of the colonies studied. This study confirms that of Bermejo & Fernández (1997) who reported that *N. apis* and *V. destructor* co-existed in the same honeybee colony even though their populations grow independently. This study also confirmed the report of Mariani *et al*. (2012) that there exist parasitic interactions between *V. destructor* and *Nosema* spp in colonies of *A. mellifera*. The association of *V. destructor* and *Nosema* spp. may later cause colony losses due to associated stress which may be caused by the infestation of *Varroa* mites and *Nosema* spp. spores. High variability between the number of *Nosema* spp. spores counted and the number of *V. destructor* observed could be due to seasonal variation in disease prevalence. Higes *et al*. (2010) demonstrated a high variability between *Nosema* spp. spore counts depending on sampling time.

Infestation levels of *V. destructor* and *Nosema* spp. in apiaries in Southwest Nigeria are considered relatively severe which need beekeepers' urgent attention in the zone to mitigate sudden colony collapse disorder in the region. The selection of healthy colonies from reliable sources and replication of colonies with good hygienic behavior is an efficient *Varroa* control measure (Castagnino *et al*. 2016, Khan & Gharmh 2021). This could be complemented with regular inspections and adequate hive hygiene management.

Conclusion

Nosema spp. spores were detected in all the honeybee samples with severe infestation needing urgent attention from the beekeepers in the zone. *Varroa* mites were present in 93% of the colonies in Southwest, Nigeria. Although the infestation level varied across the states but there is a need for continuous monitoring of the colonies for *Varroa* mites and *Nosema* spp. spores to mitigate colony decline. There is need to mention that it was initially challenging to get the consents of the bee farmers to give permissions to work on their colonies. However, the bee farmers' awareness and orientation programme on the justification and importance of the study was a bailout for the success of the study.

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