Determination and Comparison of *Nosema apis* and *Nosema ceranae* in Terms of Geographic and Climatic Factors

Coğrafik ve İklimsel Faktörler Açısından *Nosema apis* ve *Nosema ceranae*'nın Tespiti ve Karşılaştırması

Research Article

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ABSTRACT

Nosema species (*Nosema apis* and *Nosema ceranae*) are parasitic living beings that causing adult honey bee diseases called Nosemosis and honey bee colonies are often infected with *Nosema* species. *Nosema* species should be identified individually and should be compared with climatic and geographic factors in apiaries because of the requirements of different conditions. The aim of this study is screen *Nosema* species in the apiaries that have different climatic and geographic factors and compare the incidence of *Nosema* species according to these factors. With the fieldworks conducted within this study, 30 adult bees from each of 169 apiaries were collected and were studied in the laboratory for the detection of *Nosema* species. The incidence of *Nosema* species according to environmental and climatic structure of the apiaries were compared statistically. The difference among the selected provinces was found statistically significant in terms of *Nosema* infection levels by using one-way ANOVA test. At the result of this study, the determination of more *Nosema ceranae* infected colonies demonstrates that *Nosema ceranae*'s spread increased in honey bees and it began to take the place of *Nosema apis*. It was observed that the temperature and humidity values of the apiaries directly affect the *Nosema* spore density. The Pearson correlation's tests showed that the increase of these two factors increase *Nosema ceranae* incidence while the decrease of these two factors increase *Nosema apis* incidence. Therefore, it was observed that geographic and climatic differences affect the *Nosema* species and their infection levels.

Key Words
Nosema apis, *Nosema ceranae*, Nosemosis, Honey Bee.

ÖZET

Nosema apis ve *Nosema ceranae*, Nosemosis adı verilen ergin bal arısı hastalığına neden olan parazitik canlılardır ve bal arısı kolonileri sıklıkla *Nosema* türleri ile enfekte olmaktadır. Her iki tür de iklimsel olarak farklı koşullara ihtiyaç duyduğundan *Nosema* türlerinin ayrı olarak tespiti ile iklimsel ve coğrafik olarak bulundukları bölgelerin karşılaştırılması gerekmektedir. Bu çalışmanın amacı; farklı iklimsel ve coğrafik faktörlere sahip yerlerdeki arılıkların *Nosema* spp. açısından taranması ve bu faktörlere göre *Nosema* türlerinin insidansının karşılaştırılmasıdır. Bu çalışma kapsamında gerçekleştirilen arazi çalışmaları ile 169 arılığın her birinden 30 adet ergin arı toplanmış ve *Nosema* türlerinin tespiti için laboratuvarda incelemeye alınmıştır. Arılıkların çevresel ve iklimsel yapılarına göre *Nosema* türlerinin insidansı istatistiksel olarak karşılaştırılmıştır. Yapılan istatistiki çalışmalarda, tek yönlü ANOVA testi kullanarak iller arasında *Nosema* enfeksiyon düzeyi bakımından farkın anlamlı olduğu tespit edilmiştir. Bu çalışmanın sonucunda, *Nosema ceranae* ile enfekte olmuş kolonilerin daha fazla görülmüş olması, bal arılarında yayılımının arttığını ve *Nosema apis*'in yerini almaya başladığını göstermektedir. Arılıkların sıcaklık ve nem değerlerinin *Nosema* spor yoğunluğunu doğrudan etkilediği görülmüştür. Bu iki faktörün artış gösterdiği yerlerde *Nosema ceranae*'nın, azaldığı yerlerde ise *Nosema apis*'in yoğunluğunun arttığı Pearson korelasyon testleri ile tespit edilmiştir. Dolayısıyla coğrafik ve iklimsel farklılıkların, *Nosema* türleri ve enfeksiyon düzeyleri üzerinde etkisi olduğu görülmüştür.

Anahtar Kelimeler
Nosema apis, *Nosema ceranae*, Nosemosis, Bal Arısı.

Article History: Received: Jul 15, 2014; Revised: Sep 18, 2014; Accepted: Oct 22, 2014; Available Online: June 20, 2015.

DOI: 10.15671/HJBC.20154311200

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INTRODUCTION

Development period of honey bees can be a suitable environment for many pathogens and parasites. Therefore, many pathogens and parasites constitute disease in honey bees. Honey bee diseases are one of the most important factors that limiting production efficiency of honey bee products all over the world [1]. *Nosema* species (*Nosema apis* and *Nosema ceranae*) are parasitic living beings that causing adult honey bee diseases called Nosemosis and honey bee colonies are often infected with *Nosema* species. Whole honey bee colony members that consisting of queen bee, worker bee and drone can be infected with these species [2]. In addition, it is thought to be the main cause of honey bee winter-losses in many countries [1,3,4].

Nosema apis and *Nosema ceranae* that is included to Microsporidia show their effects in different ways. Nosemosis that occurs depending on *Nosema apis*, is one of the most common and most economically damaging disease in Apis mellifera. *N. apis* identified nearly 100 years ago and it was known as the only factor of Nosemosis seen in honey bees before the discovery of *N. ceranae* [5]. *N. apis* may cause the death of the diseased and weak honey bee colonies, but it does not cause to death by itself generally. Although this pandemic parasite does not have high virulence, it shortens the life of the worker bees and causes weakening in the colonies infected at high quantities. In addition, it causes to disorders in the digestive tract of honey bees [6,7,8].

In 1994, a new species of Microsporidia, *Nosema ceranae*, was discovered in Beijing, China and was detected to infect Apis cerana, eastern honey bee. Although *N. apis* and *N. ceranae* spores have similar morphology under the light microscope, *N. ceranae* is slightly smaller and more circular than *N. apis*. The main differences between these two species are determined by electron microscopic structure and the small subunit (16S) rRNA gene sequences. When these species are examined under an electron microscope, it was found to has 30 polar filaments in *N. apis* while *N. ceranae* has 20-23 polar filaments [9-11].

In 2005, natural infection of *Nosema ceranae* have been identified for the first time in *A. mellifera* colonies with the SSU rRNA gene sequencing method in Taiwan [12]. Today, this parasite is frequently found in *A. mellifera* colonies in Europe (including Ireland and the UK), North and South America, North Africa and Australia [8, 13-16]. These studies showed that *Nosema ceranae* began to take the place of *Nosema apis* throughout the World. Decline the incidence of *N. apis* In Western Europe and Australia reflects this situation. Therefore, it seems that *N. ceranae* has higher virulence than *N. apis* [8,17].

Nosema ceranae is a dangerous pathogen for *A. mellifera* and shows more lethal effect than *N. apis*. It is known to cause mass deaths and losses in honey bee colonies especially in strong colonies [18,19].

It was found that the climatic conditions change the infection level of *N. ceranae* and *N. apis* species. *N. ceranae* infection rate is more dominant in hot climates both in Europe and North America. It is thought to facilitate the spread of *N. ceranae* and increase rate of infection because of the increase of temperature in temperate climates. In contrast, *N. apis* prevalence is high in cold climates. In temperate climates, it is known that *N. apis* rate is low in summer months while it is higher during the winter months. An increase in infection with *Nosema* species was observed due to climatic changes during recent years [9, 18, 20]. The aim of this study is screen *Nosema* species in the apiaries that have different climatic and geographic factors and compare the incidence of *Nosema* species according to these factors. *Nosema apis* and *Nosema ceranae* that included Microsporidia show different effects in honey bees and both species need in different climatic conditions. With this purpose, *Nosema* species should be identified individually and should be compared with climatic and geographic factors in apiaries. Thus, it can be performed more effective fight against these honey bee parasites and can be achieved the reduction in honey bee deaths.

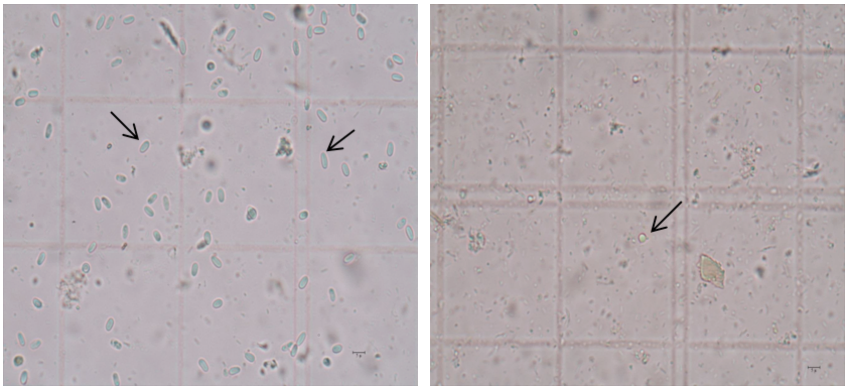

Figure 1. Microscopic views of *Nosema apis* (left) and *Nosema ceranae* (right) spores.

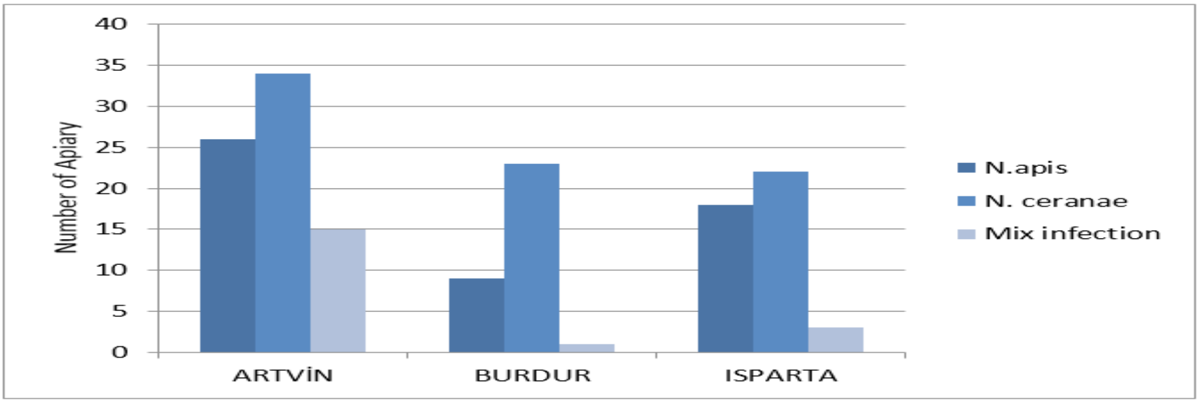

Figure 2. Distribution of *Nosema* species between the apiaries by province.

MATERIALS & METHODS

Collection the Samples of Honey Bees

In this study, apiaries that have different geographic and climatic factors both in sampling area and between sampling areas are selected and the most appropriate areas have been identified in this study. 30 adult bees from each of 169 apiaries were collected with field studies in three different regions (Artvin, Burdur and Isparta) of Turkey. Samples especially were taken from hives that showing Nosemosis symptoms. During the field studies, the locations of apiaries, altitudes and other environmental factors were measured by the GPS device and climatological data were recorded.

Isolation of *Nosema* spp. From Adult Bee Samples

The adult bees that collected by field studies were examined in the laboratory for the detection of *Nosema* species. The spore solutions were obtained by homogenizing the intestinal contents of 30 adult honey bees from each apiary for detection of *Nosema* spores and determination the level of infection in each apiary. These solutions were centrifuged at 6000 rpm for 10 minutes. After centrifugation, the supernatant was poured and the pellet was used for counting. It was added 1 ml of distilled water per adult bee on the pellet and was vortexed. These pellet solutions were used for detection of *Nosema* apis and *Nosema ceranae* spores morphologically on light microscope with Neubauer slide. In addition, infection levels per bee were determined by spore counting [21,22].

Statistical Analyzes

After biological analyzes, SPSS 14 software was used for statistical evaluation of data. Accordingly, the comparison of *Nosema* spore density in each region and between regions was performed with one-way ANOVA test and results were obtained in 95% confidence intervals. The climatic and geographic data that were measured during the field studies were compared with *Nosema* spore density using Pearson's correlation tests. The effects of these factors have been examined on a scatter plot.

Table 1. Examination of *Nosema* spp. spore concentration by provinces.

Provinces	Number of Apiary	Mean Spore Concentration	Standard Deviation	Standard Error	Minimum Value	Maximum Value
ARTVİN	81	4.1×10^6	4.2×10^6	0.7×10^6	0	24×10^6
BURDUR	38	2.5×10^6	5.2×10^6	0.8×10^6	0	25.6×10^6
ISPARTA	50	4.0×10^6	7.0×10^6	1.0×10^6	0	35×10^6

Table 2. The comparison of provinces in terms of *Nosema* spore count (One-way ANOVA, $p < 0.05$).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	920.076	66	82.340	1623	0.044
Within Groups	1567.911	102	45.467		
TOTAL	2487.987	168			

*The difference significant

Table 3. The relationship between the *Nosema* species and altitude (Pearson Correlation test).

Species	Pearson Correlation	
N. apis	**The correlation coefficient**	0.018
	P	0.901
	N	53
N. ceranae	**The correlation coefficient**	-0.216
	P	0.056
	N	79

*There is no relationship between both of *Nosema* species and altitude of apiaries

Table 4. The relationship between the *Nosema* species and temperature (Pearson Correlation test).

Species	Pearson Correlation	
N. apis	**The correlation coefficient**	-0.787*
	P	0.000
	N	53
N. ceranae	**The correlation coefficient**	0.865*
	P	0.000
	N	79

*There is positive correlation between *N. ceranae* and temperature while *N. apis* have negative correlation with temperature.

RESULTS

In this study, we determined *Nosema* species as morphologically with microscopy and we distinguished two different types of *Nosema* species with spore structure (Figure 1). After the spore determination, we detected 151 apiaries that have *Nosema* spores while there were no *Nosema* spores in 18 apiaries. In these 151 apiaries, we identified morphologically *Nosema* species and we found 53, 79 and 19 apiaries that infected with *Nosema apis*, *Nosema ceranae* and mix infection, respectively (Figure 2). In addition, *Nosema* spor densities were also determined per apiary with spore counting.

Nosema spore density comparisons were made between provinces by determining the infection levels of apiaries that have *Nosema* spores and mean, standard deviation, minimum and maximum values of each provinces were obtained (Table 1). The difference among the selected provinces was found statistically

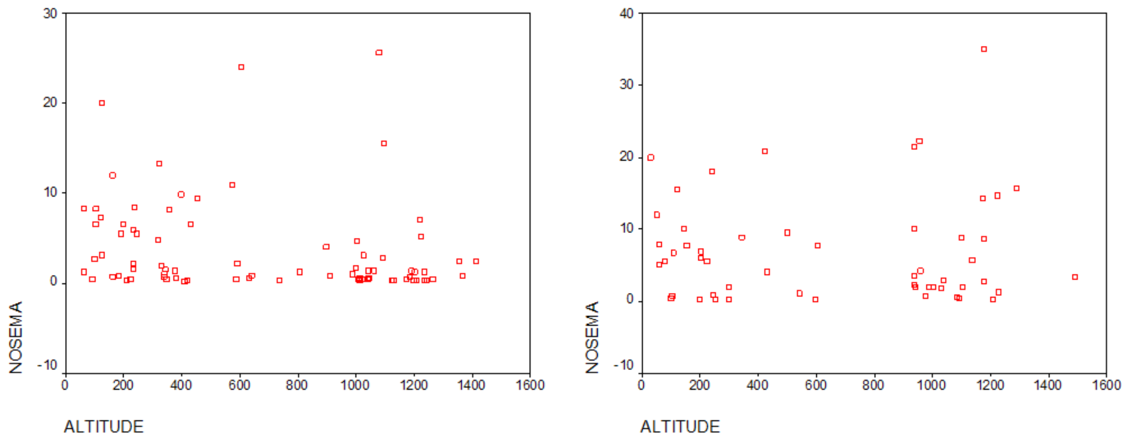

Figure 3. The scatter plot of correlation between altitudes of apiaries with *Nosema ceranae* (left) and *Nosema apis* (right).

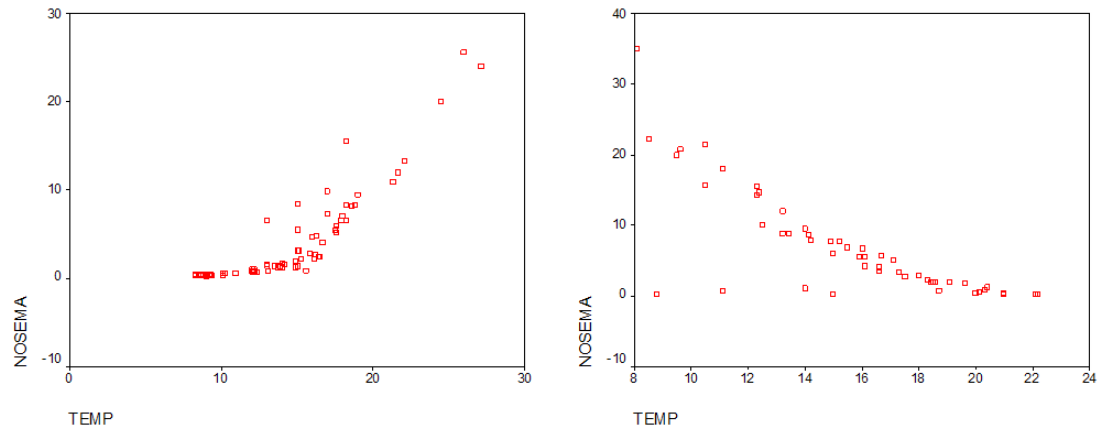

Figure 4. The scatter plot of correlation between temperatures of apiary locations with *Nosema ceranae* (left) and *Nosema apis* (right).

Table 5. The relationship between the *Nosema* species and humuditiy (Pearson Correlation test).

Species	Pearson Correlation	
N. apis	**The correlation coefficient**	-0.479*
	P	0.000
	N	53
N. ceranae	**The correlation coefficient**	0.653*
	P	0.000
	N	79

*There is positive correlation between *N. ceranae* and humidity while *N. apis* have negative correlation with moisture.

significant in terms of *Nosema* infection levels by using one-way ANOVA test (Table 2). The difference between districts of each province was also found significant with one-way ANOVA test.

Pearson correlation's test was used to see the effects of environmental and climatic changes on *Nosema* spore density. In this test, it has been compared using the altitude, temperature and humidity values. The data were supported by Scatter Plot for each factor. It was found no *Nosema apis* spore density with the altitude of apiaries (Table 3; Figure 3). When examined the effects of temperature changes on *Nosema* spore density, it was found to be direct proportion

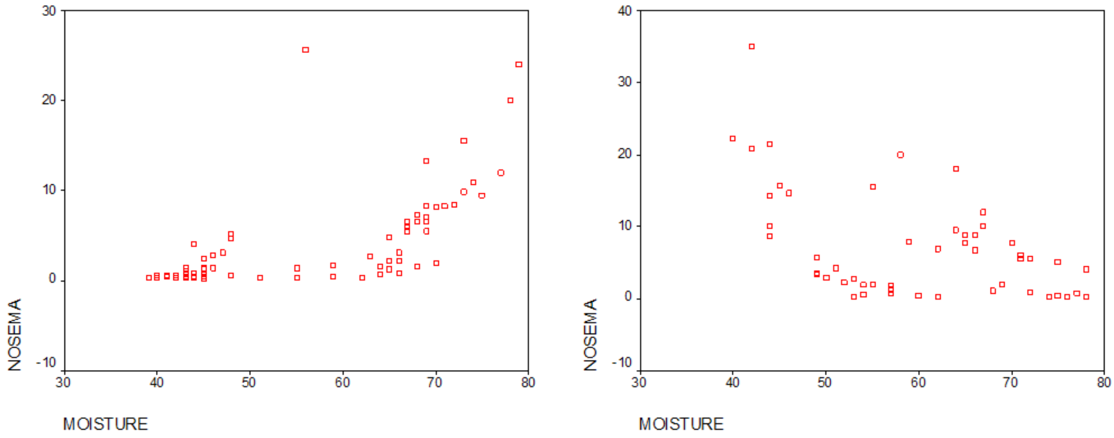

Figure 5. The scatter plot of correlation between humidity of apiary locations with *Nosema ceranae* (left) and Nosema apis (right).

Nosema ceranae spore density with tempera increase. On the contrary, it has been shown decrease in *Nosema apis* spore density with temperature increase (Table 4 and Figure 4). Humidity factor was also found to be effective on *Nosema* species. *Nosema ceranae* spore density with moisture increase was found to be direct proportion while *Nosema apis* spore density inversely proportional with moisture increase (Table 5 and Figure 5).

DISCUSSION

At the result of this study, the determination of more *Nosema ceranae* infected colonies demonstrates that *Nosema ceranae* spread increased in honey bees and it began to take the place of *Nosema apis*. One of the causes that increasing incidence of *Nosema ceranae* is able to reproduce a wider range than *Nosema apis* climatically [9]. *Nosema* infection level was found to be different among the provinces and among the districts of each province because of geographic and climatic differences between provinces and districts (Table 2). Therefore, the geographic and climatic differences have been shown to be mainly effect on *Nosema* species and infection levels of these species. These factors are examined individually and correlations between *Nosema* species and environmental factors are obtained. The absence of correlation between the altitude of apiaries and *Nosema* spore density indicates that the prevalence of the parasite is not affected by altitude (Table 3). This shows us that the geographic location of apiary is not effective by itself on the presence of parasite.

Nosema ceranae begin to appear in *A. mellifera* in recent years because of global warming and this situation suggests that there may be some factors that interact with *N. ceranae* as a cause of colony losses. Test data that obtained from Pearson correlation also confirm this situation. The data showed that *Nosema ceranae* density was increased when the temperature increases. At the same time, the presence of *Nosema apis* in apiaries where temperature decrease indicates that temperature directly affect the incidence of *Nosema* species (Table 4).

Humidity is a factor that provides a favorable environment for the reproduction of many parasites and pathogens. Moreover, humidity increases the effect of temperature more than normal and the disease agents may be seen more common. Thus, the occurrence of parasite epidemics in highly humid areas shows that climatic factors have a significant impact on the life cycle of parasites. Based on this context, the moisture levels are compared concentrations of *Nosema* species. Although *Nosema ceranae* is more intense in highly humid places, *Nosema apis* are extensively determined in apiaries that have low humidity level. This is an expected result. Favorable conditions for *Nosema ceranae* are formed by increasing the effect of temperature due to the moisture and density of *Nosema ceranae* is increased. The density of *Nosema apis* that growing with low temperatures is increased in lower humid places. Thus, the moisture level is an important factor for *Nosema* species because of its directly effect and interaction with temperature.

Nosema species is one of the most important parasites of honey bees and it is required to determine the prevalence and to identify environmental factors that affect the reproduction. The increase in the frequency of occurrence of *Nosema ceranae* revealed that the necessary measures should be taken against it. The humidity and temperature levels that determined to be directly impact on *Nosema* species are factors to be considered in the fight against these parasites. Therefore, it is necessary to focus on the dynamic relationship between the environment and *Nosema* species.

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