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First Results on the Distribution of *Nosema chaetocnemae* Yaman et Radek, 2003 (Microspora) in the Populations of *Chaetocnema tibialis* Illiger, 1807 (Coleoptera: Chrysomelidae)

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SUMMARY: In the present study, the first report on the distribution of *Nosema chaetocnemae* infection of *Chaetocnema tibialis* populations in Turkey is given. Of the 1751 beetles collected from ten provinces, 193 were infected by the parasite. The infection average was 11.02% in Turkey. *Nosema* infection was found in *C. tibialis* adults from two (Samsun and Trabzon) of the ten provinces studied. In eight localities in different regions of Turkey, the infection was not observed. The highest percentage of beetles infected with a *Nosema* isolate was recorded in Samsun. The infection average in Samsun was 25.20%. The results showed that the infection level of *N. chaetocnemae* was relatively stable during the observation period between the years 2000-2006.

Key Words: Microsporidia, parasite, distribution, *Nosema chaetocnemae*, *Chaetocnema tibialis*

Nosema chaetocnemae Yaman et Radek, 2003 (Microspora)'nın _Chaetocnema tibialis_ Illiger, 1807 (Coleoptera, Chrysomelidae) Populasyonlarındaki Dağılımı Üzerine İlk Kayıtlar

ÖZET: Bu çalışmada, *Chaetocnema tibialis* populasyonlarında *Nosema chaetocnemae* enfeksiyonunun dağılımı ile ilgili ilk bilgiler verilmektedir. Çalışma süresince on farklı lokaliteden toplanan *C. tibialis*'e ait 1751 erginin 193'ünün bu patojenle enfekte olduğu gözlenmiştir. Tüm populasyonlar için enfeksiyon ortalaması %11,02 olarak belirlenmiştir. *Nosema* enfeksiyonu örneklerin toplandığı 10 ilin ikisinde (Samsun ve Trabzon) tespit edilmiştir. En yüksek enfeksiyon %25,20 ile Samsun'da gözlenmiştir. Elde edilen sonuçlar *N. chaetocnemae* enfeksiyon seviyesinin 2000-2006 yılları arasında nispeten sabit olduğunu göstermektedir.

Anahtar Sözcükler: Microsporidia, patojen, dağılım, *Nosema chaetocnemae*, *Chaetocnema tibialis*

INTRODUCTION

Chaetocnema tibialis (Coleoptera: Chrysomelidae) is an important pest of sugar beet in Turkey. The primary damage of *C. tibialis* on sugar beet is leaf feeding by adults. The main purpose of the agricultural studies is to increase the yield of product per hectare. Leaf feeding has great effect on sugar beet growth decreasing photosynthesis capacity. Chemical pesticides utilized to control this pest should be limited because of that sugar beet leaves and residue and pulp after sugar production are used as animal nourishment. Chemical pesticides have also hazardous effects on the environment. In contrast, biological control agents have certain advantages over chemicals as control agents. As a group, Microsporidia are the most important pathogens of insects and are the most promising microorganisms for use in microbial control (13).

Despite parasites of beetles have been of great interest (15) there are a few studies on the parasite and pathogens of *C. tibialis*. Yaman and Radek (21) identified the first parasitic microsporidid, *Nosema chaetocnemae* (Microspora) from *Chaetocnema tibialis*. This is the first insect-originated *Microsporidium* identified as a new species from Turkey. Later, Yaman (20) recorded a gregarine parasite of this pest from Turkey. Additional knowledge on these parasites is of great importance and interest. Several studies on the distributions and occurrence of different *Nosema* species from different hosts have been published from different countries of Europe (6, 7, 10, 18). In the present study, a first report on the distribution and occurrence of *N. chaetocnemae* in *C. tibialis* populations between 2000 and 2006 are given.

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MATERIALS AND METHODS

Insect samples

A total of 45 localities from ten provinces (Samsun, Trabzon, Gümüşhane, Çorum, Tokat, Yozgat, Kayseri, Malatya, Balıkesir and Uşak) were randomly sampled from five different regions of Turkey between 2000 and 2006 years. Minimum 50 beetles were accepted from each locality in total for this study.

Microscopic Examination

Each beetle was usually directly dissected in a physiological solution (0.8% NaCl solution) and wet smears were examined under a microscope for parasites at the magnification of 40x to 1000x. Sometimes each beetle was placed individually into a small drop of water on a microscope slide and its body was crushed with a rounded glass stick, in order to release the spores of *N. chaetocnemae* from infected tissues (7).

The slides were air-dried and then fixed with methanol for 10 min. Afterwards the slides were washed with distilled water and stained for approximately 10 hours in a freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope (15).

RESULTS AND DISCUSSION

This is the first report on the distribution and occurrence of *N. chaetocnemae.* The first microsporidian infection in *C. tibialis* populations was observed in Samsun (Turkey) in 2000. Later, this microsporidium was identified as *Nosema chaetocnemae* (Microspora) (21). The distribution of *N. chaetocnemae* was studied adding different localities from different regions of Turkey until 2006. During the study, 1751 beetles were dissected and searched for micropsoridian infection. In total, 193 of the 1751 beetles were infected by the parasite (Table 1). Infection average was 11.02% in Turkey.

Black Sea Region	**Samsun, Çarşamba**	22.3.2000; 15.4.2000; 13.5.2000; 02.07.2000; 14.8.2000; 31.8.2000; 1.9.2000; 20.4.2001; 26.6.2001; 30.8.2001; 20.5.2003; 02.08.2005; 29.05.2006
	Trabzon	24.4.2001; 30.4.2001; 06.5.2001; 14.5.2001; 21.5.2001; 30.5.2001; 11.6.2001; 25.6.2001; 1.7.2001; 15.7.2001; 22.7.2001; 30.7.2001; 10.8.2001; 20.8.2001; 24.8.2001; 31.8.2001; 25.6.2003; 28.7.2003, 08.05.2004; 23.07.2005
	Çorum	30.8.2000, 31.08.2005
	Tokat	29.04.2004
	Gümüşhane	27.5.2001, 21.6.2001; 02.06.2006
Central Anatolia Region	**Yozgat**	26.8.2000
	Kayseri	18.7.2003
Marmara Region	**Balıkesir**	30.09.2005
Aegean Region	**Uşak**	30.09.2005
Eastern Anatolia Region	**Malatya**	04.7.2001

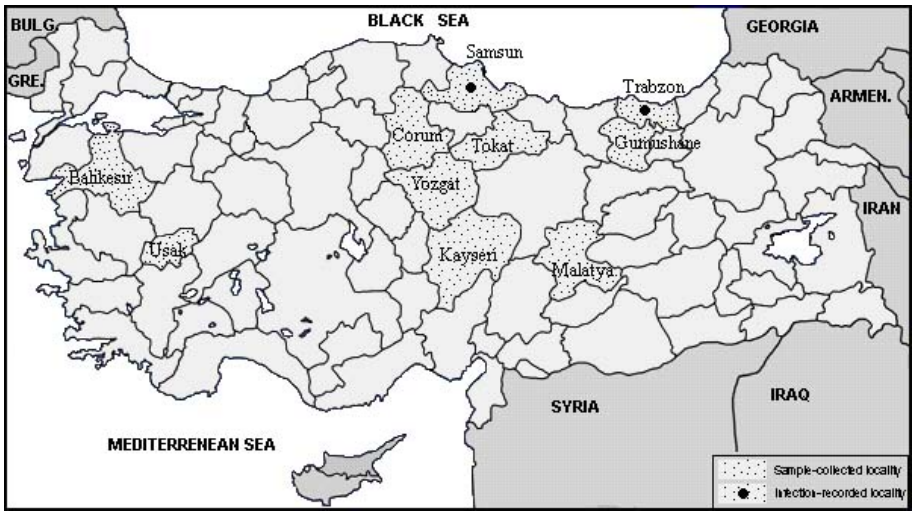

Figure 1. Prevalence of *N. chaetocnemae* in *Chaetocnema tibialis* populations in Turkey

Table 1. Occurrence of *N. chaetocnemae* in *C. tibialis* populations in Turkey in 2000-2006

Locality	Number of samples	Number of examined beetles	Number of infected beetles	Infection rate (%)
Samsun	13	718	181	25.20
Trabzon	20	366	12	3.28
Çorum	2	106	0	0
Yozgat	1	76	0	0
Tokat	1	107	0	0
Gümüşhane	3	73	0	0
Malatya	1	93	0	0
Kayseri	1	53	0	0
Balıkesir	1	75	0	0
Uşak	1	84	0	0
Total	45	1751	193	11.02

Table 2. *Nosema chaetocnemae* infections in *Chaetocnema tibialis* populations in Samsun and Trabzon in the same periods in 2001.

		Months			
		April	**June**	**August**	**Total**
Samsun	**Examined beetles**	33	49	22	114
	Infected beetles	11	15	5	31
	Infection (%)	33.3	30.6	22.7	28.1
Trabzon	**Examined beetles**	25	40	34	99
	Infected beetles	1	0	0	1
	Infection (%)	4	0	0	1.01

Microsporidian infection was found in *C. tibialis* adults from two (Samsun and Trabzon) of the ten provinces studied. The infection was not observed in Gümüşhane, Çorum, Tokat, Yozgat, Kayseri, Malatya, Balıkesir and Uşak (Figure 1). Microscopic observations revealed that the recorded micropsoridian parasite from *C. tibialis* populations in Samsun and Trabzon have typical characters of the genus *Nosema* such as spore size and morphology, the number of nuclei in the spore, diplokaryotic stages and tissue specificity (8, 9, 21).

The parasite infection was observed in two (Samsun and Trabzon) of ten provinces. 181 (25.20%) of 718 beetles from Samsun and 12 (3.28%) of 366 beetles from Trabzon were infected by the parasite. All collections (13 samples) from Samsun were infected by the parasite. However, the infection was observed in five of twenty samples from Trabzon.

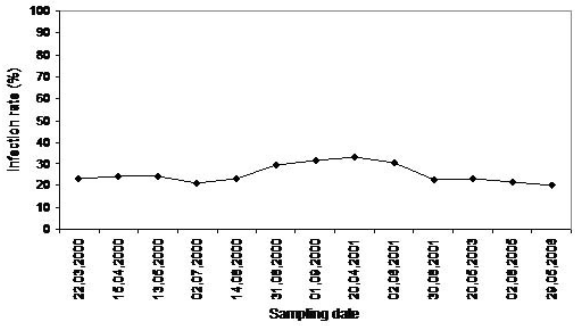

Figure 2. Infection levels of *N. chaetocnemae* in *C. tibialis* populations at Samsun in where the highest infection and all infected samples were observed in 2000-2006

The infection rate (25.20%) in Samsun is also more than that (3.28%) in Trabzon (Table 1) although both infected provinces (Samsun and Trabzon) are placed on the coast of Middle and East Blacksea Region of Turkey. The difference in the prevalence of *Nosema chaetocnemae* infection in the two provinces were found statistically significant (Khi-squre=55,96; $P<0.001$). Infection percents in the samples received from both provinces in the same periods also support this idea (Table 2). The samples from Samsun were collected on sugar beets, but that from Trabzon were collected on common vegetables because there is no sugar beet cultivation field in Trabzon. The population density of *C. tibialis* in Samsun was higher than that in Trabzon (field observation). Population density of the insect, microsporidian transmission and use of chemical pesticides effects infection rate of a microsporidian parasite in its populations. Many *Chaetocnema tibialis* individuals feed on the same sugar beet leave. The spores sometimes leave the host with the feces but are usually released after the host dies (16). The most common method of transmission is through direct oral ingestion of infectious spores found in food such as plants (2). Infection cycle commences after the spores are ingested by another host (17). This situation can cause horizontal infection of the parasite in *C. tibialis* population in Samsun. Population density of *C. tibialis* is very high in Samsun (field observation). Malone and Wigney (11) suggested that the high frequencies of infection at most sites may be a consequence of high codling moth population densities. Additionally farmers cultivating vegetable in Trabzon use chemical pesticides to control pest insects. As Rosicky (12) demonstrated, infected insects are more sensitive to chemical pesticides than healthy ones (7, 10). This situation can be a reason for the low infection level in *C. tibialis* population in Trabzon.

Nosema infection was not observed in the eight provinces from different regions of Turkey. Each region of Turkey constitutes a different climatic zone. There are a number of mountain ranges in Anatolia which constitute effective barriers against the geographical dispersal of living things (14). The studies by

Yaman et al. (22, 23) support this idea. They recorded *Nosema phyllotretae* infection in *Phyllotreta nigripens* (Coleoptera: Chrysomelidae) population from Erzurum (22) and in *Phyllotreta atra* (Coleoptera: Chrysomelidae) population from Gümüşhane (23) but they did not observe *Nosema* infection in this pest from Samsun and Trabzon.

During the study period, adults of *C. tibialis* were randomly collected from Samsun in where the highest infection was observed to detect the prevalence of the parasite between 2000-2006 years. The infection rates in the samples collected in 2000 shows the infection increases in August and September (Figure 2), but observations in other years were not enough to support this result clearly. The results showed that the prevalence of *N. chaetocnemae* was relatively stable during the observation period between 2000 and 2006 years in Samsun and that the percentage of infected beetles was relatively similar in 2000, 2001, 2003, 2005 and 2006 years (Figure 2). Zakharenkova (24) recorded that at the observation site in the Moscow region the prevalence of *Howardula phyllotretae* was relatively stable during the two-year observation period in *Phyllotreta* flea beetles. Similar results were also noted for Turkey by Yaman (19).

Although the highest infection and all infected samples were observed in Samsun, this infection would be critically enough for an effective, permanent suppression of the host. *Nosema chaetocnemae* occurred at the infection level of about 20.18-41.50% in Samsun. In four samples from Samsun the infection was observed above 30%. For pathogens such as *Nosema,* the ideal infection level would be about 30-50% (1, 7). However, Hokkanen and Lipa (7) suggest that artificial augmentation might be a solution, provided that suitable methods are found, and that compatibility of *Nosema* with pesticide treatments is achieved.

It can be concluded from this study that *Nosema chaetocnemae* dose not occur everywhere in Turkey in the populations of *Chaetocnemae tibialis*. Each region of Turkey constitutes a different climatic zone and thus accommodates different groups of plants and animals (3, 4, 5, 14, 21). Therefore, the results on the occurrence and distribution of this parasite are of great importance for the geographical distribution.

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