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Influence of climatic conditions on microsporidiosis that originated from *Rugispora istanbulensis* in Elm Leaf Beetle, *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomelidae)

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Abstract

Aim of study: This study was conducted to determine climatic conditions conducive for the spread and persistence of microsporidian infection of *Rugispora istanbulensis* in elm leaf beetle, *Xanthogaleruca luteola*.

Area of study: The samples were collected from May to September between 2013 and 2015 in İstanbul.

Material and Methods: Each month a hundred of *X. luteola* individuals were dissected and a total of 1500 beetle examined with the light microscope for three years. In this study, all statistical analyzes carried with the SPSS 21 (SPSS Inc. 1989-2012) software program.

Main results: The results indicated that the year 2014 was the most intensive year of microsporidiosis with a frequency rate of 21.48%. There was a strong correlation between the frequency of microsporidiosis and humidity ($r = 0.455$, $P<0.01$).

Research highlights: The temperature was the main determinative factor for the prevalence of microsporidiosis that originated from *R. istanbulensis* in elm leaf beetles ($t=13.671$).

Keywords: Climatic Factors, Elm Leaf Beetle, *Rugispora istanbulensis*, Microsporidiosis

Karaağaç yaprak böceği, *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomelidae)’ da *Rugispora istanbulensis* kaynaklı microsporidiosis üzerine iklim koşullarının etkisi

Özet

Çalışmanın amacı: Bu çalışma, karaağaç yaprak böceği, *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomelidae) 'da bir mikrospor türü olan *Rugispora istanbulensis* enfeksiyonunun yayılımı ve oluşumu için uygun iklim koşullarının belirlenmesi amacıyla yapılmıştır.

Çalışma alanı: Çalışma örnekleri 2013-2015 yıllarının Mayıs ile Eylül ayları arasında İstanbul’dan toplandı.

Materyal ve Yöntem: 2013-2015 yılları arasında her ay 100 *X. luteola* bireyi disekte olacak şekilde 3 yıl boyunca toplamda 1500 örnek ışık mikroskobu kullanılarak incelendi. Bu çalışmadaki tüm istatiksel analizler SPSS 21 (SPSS Inc. 1989-2012) adlı istatistik programı kullanılarak gerçekleştirildi.

Sonuçlar: 2014 yılı çalışma yılları arasında %21,48 mikrosporidiosis oranı ile en yoğun mikrospor enfeksiyonunun görüldüğü yıl olarak tespit edildi. Yapılan korelasyon analizlerine göre, nem ile microsporidiosis arasında güçlü bir korelasyon olduğu tespit edildi ($r = 0.455$, $P<0.01$).

Önemli vurgular: Karaağaç yaprak böceklerinde *R. istanbulensis* kaynaklı mikrosporidiosis yaygınlığı için sıcaklık ana belirleyici faktördür ($t = 13.671$).

Anahtar Kelimeler: İklim Faktörleri, Karaağaç Yaprak Böceği, *Rugispora istanbulensis,* Mikrosporidiosis[1]

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Introduction

Microsporidia are single-celled obligate intracellular pathogenic organisms that are rarely acute but mostly result in chronic infection (Sprague & Becnel 1999). These tiny organisms depend on their host cells to develop and reproduce. Therefore, factors that affect the host organism directly or indirectly also affect these pathogens. This is especially true for entomopathogenic microsporidia that are directly affected by the biotic and abiotic factors. Abiotic factors, especially climatic factors, play a major role in metabolic activities such as growth and development of insects (Zaslavski, 1988). For instance, as the temperature increases so does the insect activity and thereby their metabolism which plays an important role in insect growth (Wigglesworth, 1972).

In insects, microsporidia mostly cause sublethal effects on their hosts such as loss of fitness, reduced fertility, and shortened longevity (Brooks, 1988). Generally, when insect control techniques are discussed, microsporidia are considered to be important natural enemies against many pest insects. However, there are different opinions on this subject in the literature. For instance, while Canning (1982) suggests that microsporidia have an insufficient capability for use in applied biological control, Solter & Becnel (2000) suggest that microsporidia can be useful in biological control programs using inoculative release. Moreover, most studies show that microsporidia have a repressive effect on the population dynamics of pest insects (Sierpinska, 2000; Wegensteiner et al. 2015; Zhang et al., 2015). As a result, all of these studies show that microsporidia have a potential as a biological control strategy for the integrated management of pest insects. Hence, all factors affecting microsporidiosis in pest insects should be well analyzed. Particular attention should also be paid to weather and climatic conditions because weather is one of the important parameters that influences insect disease epidemics (Rahmathulla et al. 2012).

Rugispora istanbulensis Bekircan, Bülbül, Güler & Becnel, 2016 is a natural microsporidian pathogen of the elm leaf beetle, *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomelidae). The elm leaf beetle is an important pest of elm species which adversely affects urban and suburban elms in many countries like Turkey (Wu et al. 1991). Both adult and larvae stages can cause severe defoliation of elms, which can weaken mature trees even die. While adult beetles leave holes in the leaves called ‘shot-holes, larvae cause skeletonisation on a leaf, where everything is consumed except for the leaf veins (Johnson & Lyon 1991).

As for many entomopathogenic microsporidia, there are little information, and few researches examined the relationship between climatic conditions and microsporidiosis in *X. luteola*. Therefore, the present study was designed to determine which climatic conditions were conducive for microsporidiosis in elm leaf beetle.

Materials and Methods

Study location and Sample collection

Larvae and adults of *X. luteola* were collected on elm trees from May to September 2013–2015 Fatih, İstanbul (Region coordinates: 41°01′09′′ N and 28°56′14′′ E; average altitude 28 m). The beetles were put into plastic boxes and, transported to the laboratory. The beetles were dissected in Ringer’s solution and smeared on microscopic slides then observed under a binocular microscope (Olympus CX21) at magnifications of 400× for detection of *R. istanbulensis* spore structure (Bekircan et al. 2016). All dissection data were recorded for using statistical analyses. In addition, the daily climatic data included temperature, humidity, and precipitation of the study location were taken from the General Directorate of Meteorology of T.C: Ministry of Forestry and Water Affairs.

Statistical Analyzes

All data were analyzed with the SPSS 21. 0. 0.0 (SPSS Inc. 1989-2012) software program. The Kolmogorov-Smirnoff test was used to evaluate the data sets (Justel et al. 1997). Correlations and multiple regression analyses were conducted to understand the relationship between microsporidiosis and climatic factors. Correlations analysis were carried according to Spearman method (Spearman 1904).

Results

Microsporidiosis in elm leaf beetle

In the present study, a hundred *X. luteola* beetles were dissected each month between 2013 - 2015 and a total of 1500 beetle examined with the light microscope for three years. As a result, the year 2014 was determined the most intensive year of microsporidiosis with 21.48% infection rate (Figure 1).

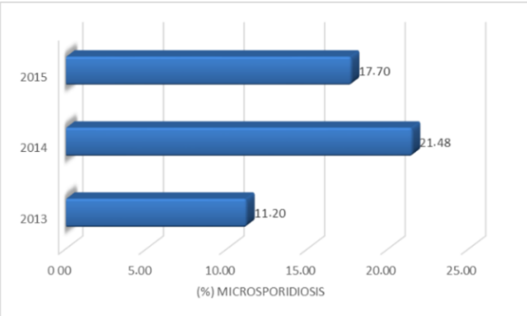

Figure 1. Microsporidiosis rates according to years

In addition, when results were evaluated according to months it was found that in 2013 and 2014 the highest microsporidiosis levels were found in August with 15.60% and 50.90% microsporidiosis rates respectively. In 2015, July had the highest prevalence rates with a 33.90% microsporidiosis rate. There were differences by year over the three years of sampling, but the months of May and June were determined to have the lowest rates of infection (Figure 2).

Figure 2. Microsporidiosis rates according to months

Climatic conditions

In the present study, climatic data for the sampling months such as temperature, humidity, and precipitation were analyzed according to months and years. During the year 2013, the highest temperature of 27.8 °C was recorded in the month of July with the lowest temperature (15.8 °C) recorded in May and the average for was determined to be 23.08 °C ± 2.94 °C. In the same year, the highest humidity was recorded in May at 80.40% relative humidity and the lowest humidity was recorded in July at 43.60% relative humidity rate (Average relative humidity: 63.69 ± 8.23%). The total highest precipitation was recorded in June at 67.40 millimeters per square meter (mm/m^2) and during the study the average precipitation was determined to be 23.48 mm/m^2 (Figure 3).

In 2014, maximum relative humidity was recorded in May at 99.99% similar to the 2013 year and the lowest relative humidity of 51.20% was recorded in the same month. The average relative humidity was determined to be 70.88 ± 8.35%. The total highest precipitation was recorded in September at 135.80 mm/m^2 (Average precipitation: 69.44 mm/m^2). The highest temperature of 27.8°C was recorded in August, the lowest temperature was in May at 13.3°C (Average temperature: 22.52 ± 3.60°C) (Figure 3).

Unlike the other two years, the highest relative humidity in 2015 was in September with 99.99% moisture rate. The lowest relative humidity was recorded in July at a 51.10% relative humidity rate (Average humidity: 70.24 ± 8.23%). The maximum temperature of 28.8°C was in July, the minimum temperature was in May at 12.9°C (Average temperature: 22.86 ± 3.18°C). The total highest precipitation was recorded in September at 37.70 mm/m^2 (Average precipitation: 19.62 mm/m^2).

Association between climatic factors and microsporidiosis

The statistical analyses showed that there was a significant relationship between climatic factors and prevalence of microsporidiosis in elm leaf beetle. A positive correlation was observed between the average monthly microsporidiosis rate and climatic factors (humidity, temperature, and precipitation). The correlation coefficient data indicated that the strongest correlation was observed between humidity and microsporidiosis (r = 0.455, P<0.01) with temperature having the weakest correlation (r = 0.256, P<0.01) (Table 1).

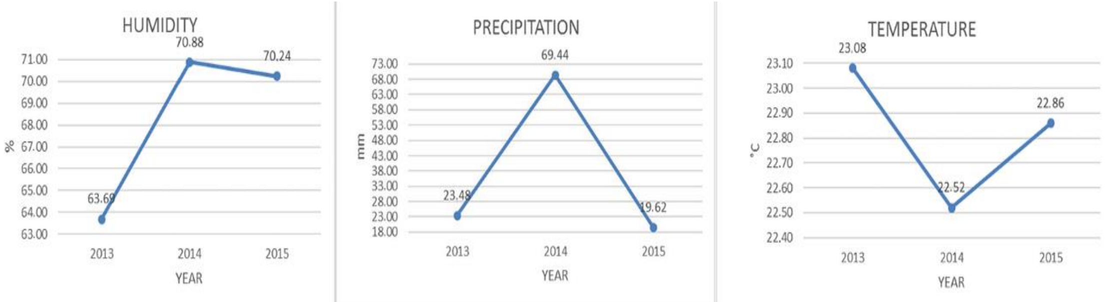

Figure 3. Mean values for different climatic conditions according to years

Multiple regressions analysis revealed that 53% of microsporidiosis formation in elm leaf beetle was correlated with climatic factors (P<0.01). The relationship between microsporidiosis and climatic factors was formulated as F (3.248) = 93.985; P<0.01. When the effects of the climatic factors on microsporidiosis were determined individually, the formula Microsporidiosis = 0.24 Humidity + 7.058 Precipitation + 4.481 Temperature - 90.179 was obtained. Furthermore, contrary to the correlation analysis, it was determined that the most influential factor on microsporidiosis was temperature (t=13.671), followed by precipitation (t=11.182) and humidity (t=0.136) respectively.

Discussion

The number of studies examined the relationship between microsporidian disease and climatic conditions is very limited. Therefore, the present study was conducted to characterize climatic conditions conducive for microsporidiosis caused by *Rugispora istanbulensis*, in the elm leaf beetle. According to the analysis made in this study, a strong correlation was determined between relative humidity and *R. istanbulensis* infection and this result was consistent with previous reports. In 2012, Rahmatulla et al determined that the highest microsporidian infection was recorded in the rainy season (June- November) when the maximum humidity rate was found.

In the present study, despite a strong correlation between humidity and microsporidiosis, regression analysis revealed that temperature was the main determinant factor of microsporidiosis. Similarly, there are many previous studies that demonstrate the effect of temperature on the spread and persistence of microsporidian diseases (Malone et al. 2001; Aydın et al. 2005; Martín-Hernández et al. 2009; Fries 2010). Temperature changes may influence intensity and prevalence of microsporidian infections in several ways. For instance, Pollan (2009) reported that *Nosema lymantria* Weiser development and spore formation in host feces occurred earlier at higher temperatures. In contrast, Solter et al. (1990) reported that in the life cycle of *Ostrinia nubilalis* Hübner the microsporidium *Nosema pyrausta* Paillot could develop more quickly at a lower temperature. Based on the results of these two studies, it can be concluded that each microsporidian species requires different environmental conditions in order to complete its optimum development.

Besides this direct effect of temperature, the temperature is a significant parameter acting on the insect feeding performance. The temperature increases lead to increased feeding behavior, especially in phytophagous insects (Lemoine et al., 2014). Therefore, the amount of horizontal transmission of microsporidia increases due to the feeding rate during periods when the temperature is high. Because of this, the intensity, and prevalence of microsporidiosis increases naturally in the insect population which is supported by the results of the present study where the main determinative factor of *R. istanbulensis* infection rates was temperature.

Table 1. Spearman correlation coefficients between climatic factors and microsporidiosis in elm leaf beetle.

	Humidity	Temperature	Precipitation	Microsporidiosis
Humidity	1.000			
Temperature	- 0.146*	1.000		
Precipitation	0.726**	- 0.291**	1.000	
Microsporidiosis	0.455**	0.256**	0.421**	1.000

**, * Correlation is significant at the 0.01 level (2-tailed) and 0.05 level (2-tailed), respectively

Precipitation also plays an important role in the transmission of microsporidian infections, especially in phytophagous insect populations (Bakk 2016). Feces of phytophagous insects infected with microsporidia contain large numbers of environmental spores and contribute to horizontal transmission (Steyer, 2010). Raindrops can lead to improved adherence of fecal particles and thus to higher contamination of plants (Goertz and Hoch 2008). In addition, raindrops carry the environmental spores from the upper parts to the lower part of plants. This situation could increase the prevalence and intensity of microsporidiosis in insect populations. Similar to these previous reports, this study found that precipitation was the second main factor after temperature affecting *R. istanbulensis* infection rates. Consequently, climatic factors can play a significant role in microsporidiosis of insects, but the dynamics of the effects can vary from microsporidian species to microsporidian species as well as the hosts and ecosystems involved.

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