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The Effects of Temperature and Humidity around the Beehives on the Distribution of *Nosema ceranae*, and also Geographical and Seasonal Activity of the Infection in the Eastern Black Sea Region of Turkey

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Abstract: 20 localities were randomly selected in Eastern Black Sea Region of Turkey and samples were collected from around the beehives from April to September. Total of 4,640 dead adult worker bees were examined during the study. Total infection rate in worker bees was 21.23%. *Nosema ceranae* was identified in all localities with molecular techniques. Temperature and humidity values were measured from around the beehives during field studies. The infection rate of *N. ceranae* increased proportionally with increasing temperature and humidity factors. Humidity was more effective than temperature on the infection rate of *N. ceranae.* The seasonal activity of *N. ceranae* was studied. The highest infection rates were observed in June and July. *N. ceranae* infection rate was higher in localities that were in low-altitude than in localities that were in high-altitude.

Key words: Temperature, humidity, geographical distribution, *Nosema ceranae, Apis mellifera*, Turkey.

1. Introduction

Apis mellifera is economically the most important bee for beekeeping in the world wide [1]. Turkey is one of the leading countries in the world in terms of honey production, despite Turkey has low levels in honey production per hive [2, 3]. One of the biggest reasons is diseases that directly affect the health of bees. In order to control these illnesses, disease factors must be examined well.

For several decades, *Nosema apis* was only known causal agent of Nosemosis in western honey bee *Apis mellifera* [4]. However, later studies have showed that *N. ceranae*, originally found in the Eastern honey bee *Apis cerana* [5], infects European honey bee *A. mellifera* [6, 7].

Black Sea Region has a significant percentile approximately 50% in terms of honey production in Turkey [2, 3]. There are few studies about the Nosemosis disease in Turkey. Especially, there are no studies on the resistance of temperature and humidity factors, additionally the seasonal distribution of *N. ceranae* infection in Turkey.

Knowing the effect of environmental factors on disease factor would provide important information for the control of disease. Temperature and humidity around the beehives were the most important environmental conditions that cause quickly spread of the infection in honeybees. In this study, temperature and humidity factors around the beehives at the natural environment were studied through the effect on the distribution of *N. ceranae* infection. Additionally, seasonal activity and geographic distribution of *N. ceranae* infection were reported in Eastern Black Sea Region of Turkey for the first time.

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2. Materials and Methods

2.1 Sample Collection

20 different localities randomly in 7 different provinces from the Eastern Black Sea region of Turkey were determined (Table 1). The adult dead worker bees were collected in front of the hives from April to September in 2011. The humidity and temperature meterdevices were placed around the bee hives. 3 times a day, temperature and humidity data were determined on a daily basis.

Samples were dissected in Ringer's solution. Wet smears were examined under a light microscope at a magnification of 400-1,000×. Spore purification and PCR amplification conditions

Positive samples were collected in sterile 1.5 mL micro centrifuge tubes. Samples micro centrifuge tubes were completed to 1 mL with distilled water. The filtered suspension was centrifuged for 6 min at 3,200 rpm. The suspension was rinsed with 1 mL distilled water. Spores were counted with a hemocytometer (2.8-5.3 × 10^7/mL). Purified spores were stored at -20 ºC until DNA extraction process [8].

Total 20 suspension samples were collected in amicro centrifuge tube for each locality and prepared for molecular characterization procedures. DNA was extracted according to the procedure [6, 9, 10] using the "DNeasy Blood & Tissue Kit" (QIAGEN, Cat. no. 69504). 50 µL PCR reaction mixture was prepared using "Qiagen Multiplex PCR Kit" (QIAGEN, Cat. no. 206143). PCR was performed following the procedure previously described by Ref. [9]. All the primer sets: 321 APIS FOR (5'-GGGGGCATGTCTTTGACGTACTATGTA-3'), and 321 APIS REV (5'-GGGGGGCGTTTAAAATGTGAAAC AACTATG-3') (*N. apis,* 321 bp fragment of the 16S rDNA) [9]; 218 MITOC FOR (5'-CGGCGACGATGTGATATGAAA-ATATTAA-3') and 218 MITOC REV (5'-CCCGGTCATTCTCAAACAAAA-AACCG-3') (*N. ceranae* 218-219 bp region of the 16S rDNA) [9]; NosA-F (5'-CCGACGATGTGATATGAGATG-3') and NosA-R (5'-CACTATTATCATCCTCAGATCATA-3') (*N. apis* 209 bp fragment of the 16S rDNA) [11]; NOS-FOR (5'-TGCCGACGATGTGATATGAG-3') and NOS-REV (5'-CACAGCATCCATTGAAAACG-3') (*N. apis* 240 bp fragment of the 16S rDNA) [6] were used one by one for PCR.

PCR was programmed as activation step of 2 minutes at 94 °C, followed by 10 cycles of 15 seconds at 94 °C, 30 seconds at 61.8 °C, and 45 seconds at 72 °C, and 20 cycles of 15 seconds at 94 °C, 30 seconds at 61.8 °C, and 50 seconds at 72 °C plus a 5-second elongation cycle for each successive cycle and a final extension step at 72 °C for 7 minutes. Negative controls (from DNA extraction) were included in all PCR reactions [9]. The molecular weights of PCR products were determined by electrophoresis in a 0.9% agarose gel stained with ethidium bromide, and visualized using UV illumination.

3. Statistical Analysis

Between 2009 and 2011, total of 102 times locality study was performed. In this study, in all locality data of Nosema disease infection were obtained according to the provinces, the difference the altitude of localities, temperature change and humidity change around the beehives. The data obtained in this study were compared with correlation and regression analysis using SPSS 11.0 software [12].

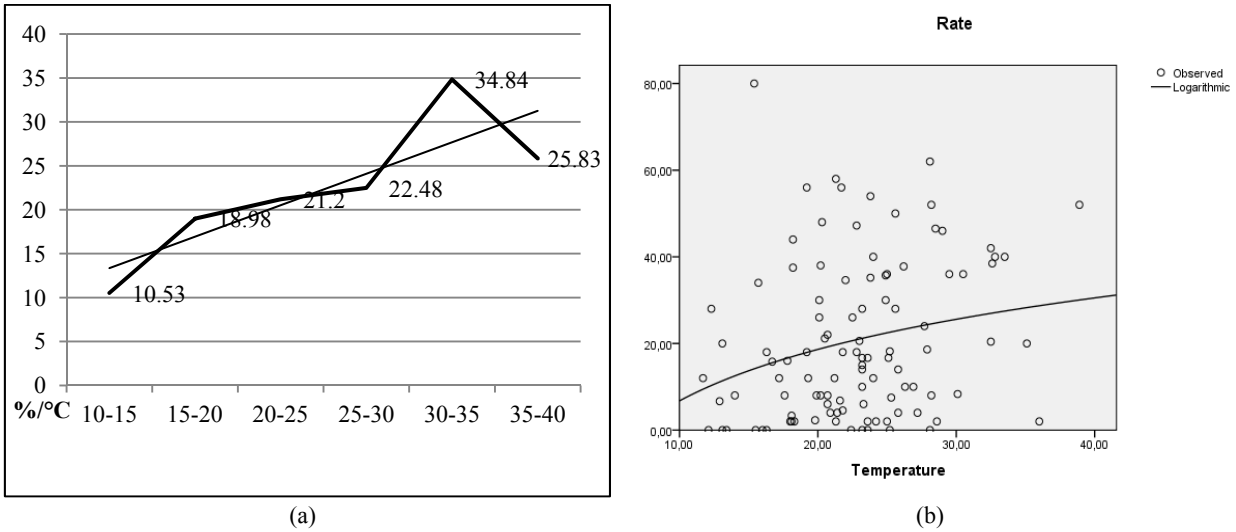
4. Results

Worker bees were examined in Eastern Black Sea of Turkey in 2011. 985 of 4,640 (21.23%) dead worker bees which infected with *N. ceranae* were observed. Temperature and humidity data were reported separately for each locality.

While the lowest temperature datum was determined as 11.7 ºC in May in Gümüşhane center localitythat it has 12% infection rate, the highest temperature datum was determined as 38.9 ºC in of locality that infection rate was 52% in July (Table 1). When temperature was in the range of 11-15 ºC, average *N. ceranae* infection rate was around 10%. With increasing temperature (the range of 15-30 ºC), infection rate showed an increase and was observed about 18%-22%. When the temperature increases in rage of 30-35 ºC, infection rate reached average 35%. Different from the other temperature ranges, infection rate started to decrease and in decreased rate of 25% in the range of 35-39 ºC (Table 1, Fig. 1).

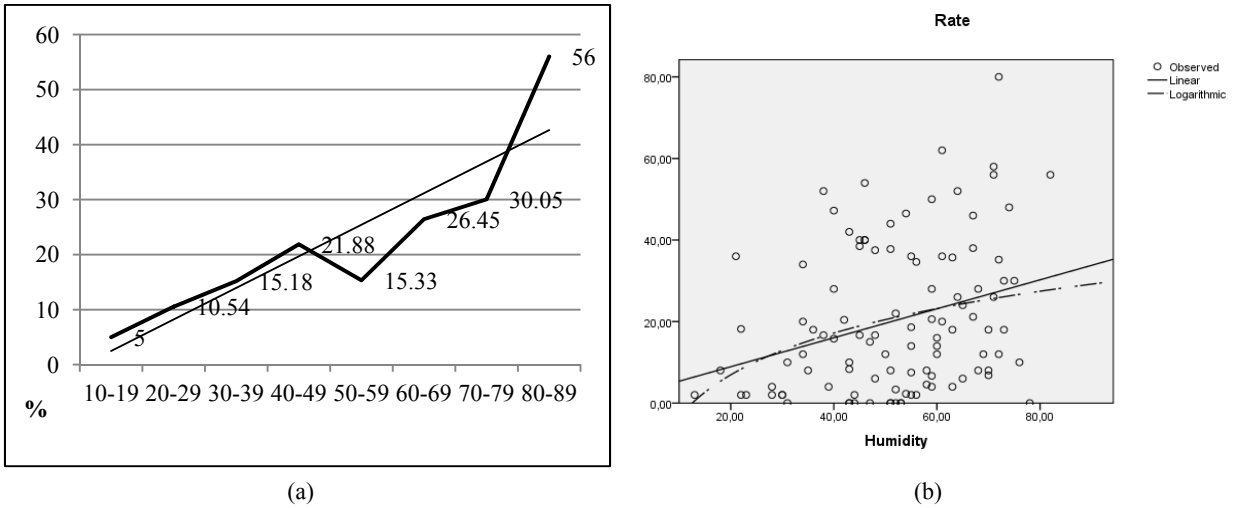
According to data from humidity, the lowest humidity datum was observed as 13% in Alucra locality in July. And also, infection rate was 2% in that locality. Additionally, 82% was determined as the highest humidity rate in Ulubey locality where infection rate was 56% in June (Table 1). While humidity was in the range of 13-19%, average infection rate was observed 5%. The infection rates were showed increase with the humidity rates proportionally. When the humidity raised from 20% to 49%, average infection rate reached from 5% to 21%. Different from other humidity data, in the range of 50%-59% humidity, average infection rate decreased at 15%. Humidity rates increased from 60% to 82%, the infection rates increased again and it reached as high as 56% (Table 1, Fig. 2).

Geographical distribution of *N. ceranae* infection was studied according to the localities in different altitude. Depending on the geography of the Eastern Black Sea Region of Turkey 0-1,000 m and 1,000-2,000 m altitudes were interpreted. The total infection in high altitude localities was found to be 17.24%, the total infection in low altitude localities was higher than in high altitude localities, and also it was 24.55% (Table 1). Each selected two areas in the province of Artvin were under 1,000 m altitude, and total infection rate in Artvin province was 33.33%. For Rize province, there was a different situation between the infection rate (26.57%) in high altitude localities (Ayder and Anzer localities) with the infection rate (20.5%) in low altitude localities (Rize center and Pazar localities). In Trabzon province, the average infection rate observed as 20.13% in high altitude localities (Uzungöl and Tonya localities) was higher than the average infection rate observed as 14.35% in low altitude localities (Trabzon center, Of and Beşikdüzü localities), this situation of Trabzon province was similar with that Rize province (Table 1). The average infection rates in high altitude localities



(a) (b)

Fig. 1 The average *N. ceranae* infection rates according to changes in temperature.
Figures were made with Microsoft Excel (A) and SPSS 11.0 software (B) programs.



(a) (b)

Fig. 2 The average *N. ceranae* infection rates according to changes in humidity.
Figures were made with Microsoft Excel (A) and SPSS 11.0 software (B) programs.

Table 1 Distribution of *N. ceranae* in *A. mellifera* in Eastern Black Sea region of Turkey in 2011.

Localities		Coordinates		Collection date	Temperature	Humidity %	Number of insects worker bees		
							Dissected	Infected	%
Artvin	Hopa	La.	41.4333	11.04.2011	13.1	61	50	10	20.00
				26.05.2011	23.6	38	18	3	16.67
		Lo.	41.4702	27.06.2011	20.3	74	50	24	48.00
				25.07.2011	28.2	64	50	26	52.00
		Al.	185 (m)	22.08.2011	28.5	54	43	20	46.51
				24.09.2011	22.4	53	26	0	0.00
	Arhavi	La.	41.3351	11.04.2011	12.9	59	60	4	6.67
				26.05.2011	22.8	40	36	17	47.22
		Lo.	41.3028	27.06.2011	21.7	71	50	28	56.00
				25.07.2011	29	67	50	23	46.00
		Al.	76 (m)	22.08.2011	25.6	68	50	14	28.00
				24.09.2011	23.2	52	24	0	0.00
Rize	Ayder	La.	40.9514	11.04.2011	-	-	-	-	-
				23.05.2011	15.4	72	55	44	80.00
		Lo.	41.1185	27.06.2011	25.6	59	50	25	50.00
				28.07.2011	26.3	43	50	5	10.00
		Al.	1385 (m)	22.08.2011	17.8	60	50	8	16.00
				24.09.2011	19.9	70	50	4	8.00
	Pazar	La.	41.1707	11.04.2011	13.1	51	26	0	0.00
				23.05.2011	20.5	67	52	11	21.15
		Lo.	40.88	27.06.2011	20.1	75	50	15	30.00
				25.07.2011	32.6	45	26	10	38.46
		Al.	199 (m)	22.08.2011	30.1	43	12	1	8.33
				29.09.2011	18.1	52	30	1	3.33
	Center	La.	41.0279	11.04.2011	14	58	25	2	8.00
				23.05.2011	20.2	67	50	19	38.00
		Lo.	40.4954	27.06.2011	19.2	70	50	9	18.00
				25.07.2011	27.7	65	50	12	24.00
		Al.	204 (m)	22.08.2011	27.9	55	43	8	18.60
				29.09.2011	17.6	51	25	2	8.00

Table 1 to be continued

Rize	Anzer	La.	40.6262	11.04.2011	-	-	-	-	-
				23.05.2011	16.7	40	38	6	15.79
		Lo.	40.5458	27.06.2011	12.3	59	50	14	28.00
				25.07.2011	23.2	60	50	7	14.00
		Al.	1991 (m)	23.08.2011	23.2	40	50	14	28.00
				29.09.2011	20.2	35	50	4	8.00
Trabzon	Uzungöl	La.	40.633	11.04.2011	-	-	-	-	-
				27.05.2011	21.8	58	22	1	4.55
		Lo.	40.2851	30.06.2011	20.7	52	50	11	22.00
				28.07.2011	25	55	50	1	2.00
		Al.	1109 (m)	23.08.2011	15.5	78	50	0	0.00
				30.09.2011	18.1	44	50	1	2.00
	Of	La.	40.8958	11.04.2011	-	-	-	-	-
				27.05.2011	20.1	64	50	13	26.00
		Lo.	40.2709	30.06.2011	22.8	63	50	9	18.00
				28.07.2011	38.9	38	50	26	52.00
		Al.	201 (m)	23.08.2011	21.8	73	50	9	18.00
				30.09.2011	21.4	59	50	2	4.00
	Center	La.	40.9796	11.04.2011	-	-	-	-	-
				27.05.2011	17.2	72	50	6	12.00
		Lo.	39.7631	27.06.2011	23.2	76	20	2	10.00
				28.07.2011	28.1	43	50	0	0.00
		Al.	300 (m)	27.08.2011	25.8	55	50	7	14.00
				30.09.2011	16.3	51	30	0	0.00
	Beşikdüzü	La.	40.971	11.04.2011	-	-	-	-	-
				27.05.2011	21.6	70	44	3	6.82
		Lo.	39.2462	30.06.2011	23	59	34	7	20.59
				28.07.2011	35.1	34	20	4	20.00
		Al.	419 (m)	24.08.2011	25.3	55	40	3	7.50
				30.09.2011	23.6	53	46	0	0.00
	Tonya	La.	40.7991	11.04.2011	-	-	-	-	-
				27.05.2011	23.8	46	50	27	54.00
		Lo.	39.2722	30.06.2011	21.3	71	50	29	58.00
				29.07.2011	30.5	55	50	18	36.00
		Al.	1257 (m)	24.08.2011	21.2	60	50	6	12.00
				30.09.2011	18.3	56	50	1	2.00
Giresun	Espiye	La.	40.9553	11.04.2011	-	-	-	-	-
				24.05.2011	28.1	61	50	31	62.00
		Lo.	38.6578	28.06.2011	24.9	73	50	15	30.00
				26.07.2011	33.5	46	50	20	40.00
		Al.	45 (m)	24.08.2011	26.2	51	45	17	37.78
				27.09.2011	20.7	65	50	3	6.00
	Tirebolu	La.	40.9982	11.04.2011	-	-	-	-	-
				24.05.2011	24.9	63	28	10	35.71
		Lo.	38.81	28.06.2011	23.8	72	54	19	35.19
				26.07.2011	29.5	61	50	18	36.00
		Al.	187 (m)	24.08.2011	25.2	47	50	0	0.00
				27.09.2011	20.7	68	50	4	8.00

Table 1 to be continued

Giresun	Alucra	La.	40.3163	11.04.2011	-	-	-	-	-
				24.05.2011	18.2	51	50	22	44.00
		Lo.	38.7684	23.06.2011	25	21	50	18	36.00
				27.07.2011	36	13	50	1	2.00
		Al.	1496 (m)	20.08.2011	28.2	18	50	4	8.00
				26.09.2011	18	28	50	1	2.00
	Şebin karahisar	La.	40.3634	11.04.2011	-	-	-	-	-
				24.05.2011	19.8	54	44	1	2.27
		Lo.	38.5824	23.06.2011	24.2	30	50	1	2.00
				27.07.2011	26.9	31	50	5	10.00
		Al.	1630 (m)	20.08.2011	25.8	28	50	2	4.00
				26.09.2011	16	31	26	0	0.00
Ordu	Ulubey	La.	40.8548	11.04.2011	-	-	-	-	-
				25.05.2011	22	56	52	18	34.62
		Lo.	37.7831	29.06.2011	19.2	82	50	28	56.00
				26.07.2011	32.5	43	50	21	42.00
		Al.	497 (m)	27.08.2011	23.2	47	20	3	15.00
				27.09.2011	24	50	50	6	12.00
	Gürgentepe	La.	40.7925	11.04.2011	-	-	-	-	-
				25.05.2011	18.2	48	24	9	37.50
		Lo.	37.5199	29.06.2011	19.3	69	50	6	12.00
				26.07.2011	32.5	42	49	10	20.41
		Al.	1115 (m)	27.08.2011	25.1	45	48	8	16.67
				27.09.2011	23.2	48	30	5	16.67
	Perşembe	La.	41.0857	11.04.2011	-	-	-	-	-
				25.05.2011	24	46	50	20	40.00
		Lo.	37.6479	29.06.2011	22.5	71	50	13	26.00
				26.07.2011	32.8	45	50	20	40.00
		Al.	182 (m)	27.08.2011	23.3	48	50	3	6.00
				27.09.2011	20.9	63	50	2	4.00
Gümüşhane	Gümüşhane Center	La.	40.4865	11.04.2011	-	-	-	-	-
				28.05.2011	11.7	34	50	6	12.00
		Lo.	39.5549	23.06.2011	25.2	22	44	8	18.18
				27.07.2011	28.6	23	50	1	2.00
		Al.	1744 (m)	20.08.2011	23.6	22	50	1	2.00
				28.09.2011	13.4	44	50	0	0.00
Bayburt	Demirözü	La.	40.2011	11.04.2011	-	-	-	-	-
				28.05.2011	15.7	34	50	17	34.00
		Lo.	39.8585	30.06.2011	16.3	36	50	9	18.00
				27.07.2011	27.2	39	50	2	4.00
		Al.	1675 (m)	20.08.2011	21.3	30	50	1	2.00
				28.09.2011	12.1	43	31	0	0.00
Total							4640	985	21.23

La: Latitude, Lo: Longitude, Al: Altitude.

were lower than that in low altitude localities for Giresun and Ordu provinces being different from Rize and Trabzon provinces. In Giresun province, the average infection rate in high altitude localities (Alucra and Şebinkarahisar localities) was 11.7% and the average infection rate in low altitude localities (Tirebolu and Espiye localities) was 28.72%. Similarly, in Ordu province, the average infection rates in high altitude

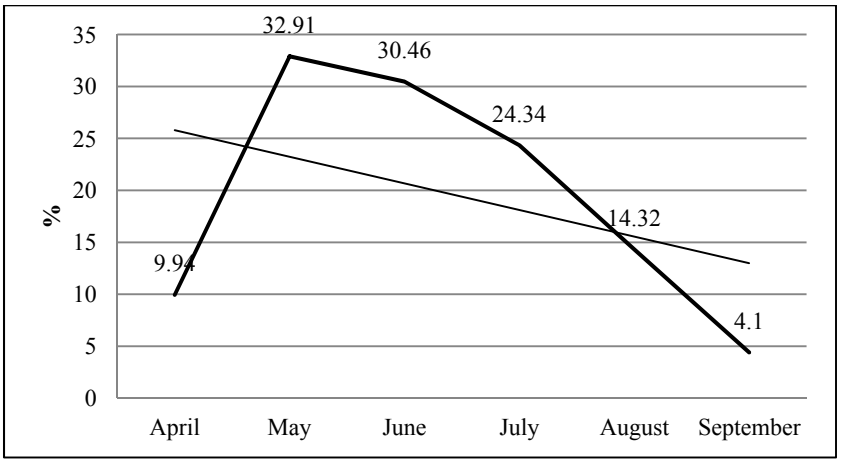


Fig. 3 The average *N. ceranae* infection rates according to changes in month.

localities (Gürgentepe locality) and in low altitude localities (Perşembe and Ulubey localities) were 18.9% and 28.38% respectively (Table 1). Gümüşhane center locality in Gümüşhane province and Demirözü locality in Bayburt province were directly over 1,000 m altitude localities and also infection rates were 6.5% and 12.5% respectively (Table 1).

N. ceranae infection rates of the examined samples showed remarkable difference from April to September. Nosemosis infection was found in every month of the six-month process. The infection rate was 9.93% in April. The highest infection rate was observed in May as 32.91%. The average *N. ceranae* infection rates decreased from May to September, and also infection rates were 30.46%, 24.34% 14.32% and 4.4% respectively (Table 1, Fig. 3).

5. Discussion

In beekeeping, honey bees are affected by many kinds of environmental factors such as temperature and humidity or geographical and seasonal variations in their habitats. Besides, the pathogen and parasites of honey beesare are also affected by these environmental factors.

It can be said that for this study, Nosemosis infection significantly is affected by temperature change. Infection is directly proportional to temperature around the beehives (Pearson correlation, $P < 0.05$, $r = 0.245$). References [7, 12] reported that temperature was a significant factor in the presence of Nosemosis infection. *N. ceranae* infection is more resistant to temperature changes than *N. apis* [13-15] reported that in his study in the laboratory conditions, *N. ceranae* spores did not lost infection activity from 4 ºC to 60 ºC, but *N. apis* spores had less temperature tolerance. Higes et al. [16] reported that the both agent of nosemosis disease shows 99% vitality at 33 ºC, and also between 25 ºC and 37 ºC temperatures. *N. ceranae* shows more vitality than *N. apis*. In this study, the most infection rate observed between 30 ºC and 35 ºC temperatures for *N. ceranae*. Similarly in this study a very high rate of infection were determined at 30 ºC temperature around the beehives. Hive temperature is around 35 ºC and this temperature is ideal for the survival of both disease agents [15]. In this study, when the temperature around the beehives was above 35 ºC, decrease in the rate of infection was determined. Malone et al. [17] reported that Nosema spores that cause Nosemosis disease are able to continue the viability in the bee stool and cadavers in front of the hive, additionally temperature and humidity are important factors in contamination other bees Nosema infection.

In this study, ıt can be said that humidity has effect on nosemosis infection distribution likewise temperature. Infection is directly proportional to humidity around the beehives (Pearson correlation, $P < 0.05$, $r = 0.295$). It is known that increased rainfall

increases the efficiency of humidity. Aydin et al. [12] stressed that the rainfall was an important factor on Nosema infection. Malone et al. [17] reported that *N. apis* lost the viability of infection in dry weather between 40 ºC and 49 ºC within 3 to 45 days. It is discussed that not only hashumidity effect on the viability of Nosemosis spores but also it increases the spread of Nosema spores and the capacity of transmitting infection to other bees [12, 14, 17].

While temperature was at low level, humidity was high and infection rate was also high in some localities for example infection rate was 80% in Ayder locality in May. Additionally, while humidity was low, temperature was at high level and infection rate was also high in the some localities for example infection rate was 2% in Alucra locality in July. It can be said that humidity was more an effective factor than temperature on distribution of *N. ceranae*. While humidity is stationary, the correlation between the temperature and the infection was found to be 0.347 (Pearson correlation, $P < 0.05$, $r = 0.347$). Additionally, while temperature is stationary, the correlation between the humidity and the infection was found to be 0.381 (Pearson correlation, $P < 0.05$, $r = 0.381$). In this case, it can be interpreted that the power of the linear relation between infection with humidity is slightly more effective than the power of the linear relation between temperature and infection. Aydin et al. [12] reports that rainfall and humidity factors, are more important factors than the temperature on infection Nosemosis. Climate changes effect on the distribution, the radiation and the presence of disease organisms in the insects [14, 18]. *N. ceranae* is more tolerant of the climate change compared to *N. apis*, Whereas *N. ceranae* showed prevalence in warm climate countries, and *N. apis*is more common in colder climates countries [19].

Detection of climatic changes plays an important role in the identification and in the struggle for Nosema disease of honey bees [20]. *N. ceranae* infection rate in the areas with high altitude was lower than level of infection in the areas with low altitude (Chi-Square, $p < 0,05$, $\chi2 = 24,057$, $df = 1$). The reason for this is that temperature and humidity data were variable in different altitude localities. While temperature data were close rates, there is a high difference between humidity data in low and high altitude areas. The low humidity was one of the main reasons for low levels of infection in high altitude areas.

Nosemosis infection was found in all months the process study was conducted. Infection was at very low levels in April compared to other months. The main reasons for this is that bee colonies have winter conditions due to unfavorable weather conditions in Eastern Black Sea Region in April, 2011. As for the May, in which the infection rate is at quite high level shows an increasing decrease straight accurate month of August (Chi-Square, $p < 0.05$, $\chi2 = 240,04$, $df = 5$). The temperature and humidity factors are thought to play an important role in Nosemosis infection showing the variable results according to months. While the average temperature showed an increase, humidity declined in average between April and September in 2011. Both the temperature and humidity to decline after July were effective on decline of infection rate. In the literature, several studies reported that *N. ceranae* infection increased from April to June but decreased from September to March. In the opposite case, *N. apis* infection especially increased in winter months and losed its volume in summer months [21-23] studied in Virginia and Germany respectively and they reported that *N. ceranae* infection had a very high level in late spring and early summer, but it had been go down to a low level during the winter season. In a different situation, Martín-Hernández et al. [14] reported in their study in Spain, *N. ceranae* infection did not show a seasonal activity. *N. ceranae* especially showed activity during the summer months but *N. apis* showed activity during the winter months, because of that *N. ceranae* was more tolerant the temperature from *N. apis* [14, 21]. In

many studies reported that *N. ceranae* seen activity in all seasons, especially it had higher levels in the summer. References [9, 14, 21, 24] discussed in their study that *N. ceranae* infection quickly replace to *N. apis* infection, because *N. ceranae* infection was more intense than *N. apis* in summer. Additionally, Brenna et al. [21] said that one-year period study was sufficient seasonal activity work.

The effects of temperature and humidity around the beehives on the distribution of *N. ceranae* infection, and its geographical and seasonal activity in the Eastern Black Sea region of Turkey was studied in this study for the first time.

6. Conclusion

The effects of temperature and humidity around the beehives on the distribution of *N. ceranae* infection, and its geographical and seasonal activity in the Eastern Black Sea region of Turkey was studied in this study for the first time.

Worker bees were examined in Eastern Black Sea of Turkey in 2011. 985 of 4,640 (21.23%) dead worker bees which infected with *N. ceranae* were observed. Temperature and humidity data were reported separately for each locality.

While the lowest temperature datum was determined as 11.7 ºC in May in Gümüşhane center locality that it has 12% infection rate, the highest temperature datum was determined as 38.9 ºC in of locality that infection rate was 52% in July.

According to data from humidity, the lowest humidity datum was observed as 13% in Alucra locality in July. And also infection rate was 2% in that locality. Additionally, 82% was determined as the highest humidity rate in Ulubey locality where infection rate was 56% in June.

The total infection in high altitude localities was found to be 17.24%, the total infection in low altitude localities was higher than in high altitude localities, and also it was 24.55%

Nosemosis infection was found in every month of the six-month process. The infection rate was 9.93% in April, The highest infection rate was observed in May as 32.91%. The average *N. ceranae* infection rates decreased from May to September, and also infection rates were 30.46%, 24.34% 14.32% and 4.4% respectively.

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