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ORIGINAL ARTICLE

FORENSIC BIOLOGY

Bursa ili Mustafakemalpaşa ilçesinde adli entomoloji açısından önem taşıyan sinek (Insecta: Diptera) faunasının araştırılması

Investigation of fly (Insecta: Diptera) fauna that is important for forensic entomology in Mustafakemalpaşa, Bursa

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ÖZET

AMAÇ: 2017 yılında yapılan bu çalışma ile Bursa ili Mustafakemalpaşa ilçesinde Adli entomoloji açısından önem taşıyan sinek faunası araştırılmıştır.

YÖNTEM: Çiftlik domuzunun üç farklı mevsimde çürüme evreleri ile domuz leşi üzerindeki ergin sinek ve larvalar toplanmıştır.

BULGULAR: Saha çalışmaları sonucunda Sarcophadigae familyasından 3 tür (Sarcophaga variegate Scopoli, 1763; Sarcophaga lehmanni Müler, 1922; Wohlfahrtia magnifica Schiner, 1862), 7 tür (Calliphora subalbina Ringdahl, 1931; Calliphora uralensis Villeneuve, 1922; Calliphora vomitoria Linnaeus, 1758; Calliphora Vicinia Robineau-Desvoidy 1830; Lucilia caesar Linnaeus, 1758; Luciliaic ampullacea Villeneuveata, 1922'den Luciliaic ailesi, 1922) Muscidae familyasından 2 tür (Musca domestica Linnaeus, 1758; Muscina stabulans Fallen, 1817) rapor edilmiştir.

SONUÇ: Calliphora vicinia, Mayıs, Temmuz ve Ekim aylarında yapılan her üç saha çalışmasında da tespit edilen en baskın türdür. Calliphoridae, Sarcophagidae ve Muscidae familyalarının üçüncü evre evreleri ve süreleri verilmiştir. Mayıs, Temmuz ve Ekim meteorolojik verileri verilmiştir.

Anahtar Kelimeler: Adli entomoloji, Çiftçilik domuzu, Bursa.

ABSTRACT

INTRODUCTION: In a study conducted in 2017, the aim was to determine the fly species that are significant in forensic entomology in Mustafakemalpaşa, Bursa.

METHODS: Decomposition phases of *Sus scrofa* (domestic pig) were examined in different seasons and site conditions.

RESULTS: As a result of field studies, three species—*Sarcophaga variegata* (Scopoli, 1763), *Sarcophaga lehmanni* (Müler, 1922), and *Wohlfahrtia magnifica* (Schiner, 1862)—from the Sarcophadigae family, seven species—*Calliphora subalbina* (Ringdahl, 1931), *Calliphora uralensis* (Villeneuve, 1922), *Calliphora vomitoria* (Linnaeus, 1758), *Calliphora vicina* (Robineau-Desvoidy, 1830), *Lucilia caesar* (Linnaeus, 1758), *Lucilia ampullacea* (Villeneuve, 1922), and *Lucilia sericata* (Meigen, 1826)—from the Calliphoridae family, and two species—*Musca domestica* (Linnaeus, 1758) and *Muscina stabulans* (Fallen, 1817)—from the Muscidae family were reported.

CONCLUSION: *Calliphora vicina* is the dominant species detected in all three field studies conducted in May, July, and October, 2017. The third instar phases and durations of the Calliphoridae, Sarcophagidae, and Muscidae families are given. Meteorological data from May, July, and October are included.

Keywords: Forensic entomology, Farm pig, Bursa.

INTRODUCTION

The essential application of forensic research is to investigate evidence found at a scene with scientific and technical methods to make a fair and objective judgment. This entails identifying the evidence, documentation, duly collecting the evidence, and analyzing the evidence by making an assessment in a laboratory. Finding the PMI (post-mortem interval) is of great importance in illuminating many unsolved incidents. Forensic entomology provides valuable evidence and contributes to the determination of time of death. From the moment a fly (Diptera species, the most

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important insect group in forensic events) lays eggs on a corpse, knowledge of its life cycles in order to determine the age and calculating estimated PMI is the job of forensic entomologists (1).

Forensic entomology is a broad discipline in which insect science and forensic systems interact (2). It is divided into three subdivisions: criminal entomology, urban entomology, and stored-product entomology (3). Criminal entomology deals with insects detected on a corpse in forensic events, urban entomology deals with the economic importance and methods of combatting insects that are directly or indirectly harmful, and stored-product entomology deals with insects that invade foods. Despite this distinction, the terms criminal entomology and forensic entomology are used interchangeably. When a human body is found, the critical questions are usually how, when, and where death occurred. PMI detection is calculated by such factors as body temperature, muscle softness, rigidity, bruises, and skin and nail pallor. Criminal entomology, on the other hand, helps estimate the time since death, often by calculating the development phase of insects that have been attracted to the body (4–7).

The most important groups used in criminal entomology are species from the Diptera, Sarcophagidae, Muscidae, and especially the Calliphoridae families. Identification of the species on or near the corpse, knowing the life cycle and behavioral patterns of that species, and providing minimum PMI calculations at the scene can provide information about the crime scene and the presence of drugs or poison in the body (3,7–19). Insects feed on the carcass in a faunal sequence at various stages of decay. Based on information on the insects invadng the body, recognition of the different immature (larval and pupal) stages of each species, and their development time can provide an estimate for PMI (7).

Regional distribution studies of Calliphoridae, Muscidae, and Sarcophagidae families, the most important groups in criminal entomology, have been conducted in North America, South America, Europe, Africa, and Australia (3,11,12,15,20–36).

Studies on this subject in our country are limited. Specifically, the fauna and distribution of insect groups that are important in our country's forensic entomology should be well known. In our country, criminal entomology studies are very new and

Table 1: Species Identified in the First Field Study

Determinations	19.05.2017	20.05.2017	21.05.2017	22.05.2017	23.05.2017	24.05.2017	25.07.2017	26.05.2017	27.05.2017	28.05.2017	29.05.2017	30.05.2017	31.05.2017	01.06.2017	02.06.2017
Lucilia ampullacea	+	+		+	+										+
Calliphora vicina	+	+		+	+										+
Muscina stabulans	+	+		+	+							+			
Calliphoridae 3.instar						+		+	+	+	+	+			
Muscidae 3.instar						+		+	+	+	+	+			

Figure 1: Stainless Iron Cage and Protective Jump Suit

remain limited (1,37–42). In 2005, Akdemir conducted a study to identify the insect species found on human corpses in the post-mortem period in Samsun province; as a result of that research, 13 insect species belonging to Diptera and Coleoptera families were identified (39).

Şabanoğlu and Sert conducted a study in Ankara Province (Central District) on the determination of the Diptera fauna on a corpse and a systematic investigation of its morphology. According to their data, four Calliphoridae species were identified on a pig carcass at the Beytepe Campus of Ankara (41).

Özdemir and Sert (2008) conducted a study to examine the Coleoptera fauna on a corpse and a systematic investigation of its morphology in the city of Ankara (Central District). They identified 40 species of the Staphylinidae, Histeridae, Dermestidae, Silphidae, Nitidulidae, and Cleridae families belonging to Coleoptera on a pig carcass (Sus scrofa L.), which was put in the test field for a year on the Beytepe Campus of Ankara.

Açıkgöz, Açıkgöz, and İşbaşar (2011) performed death-time determination with entomological evidence collected on human corpses in and around Ankara. In their study, 16 cases were evaluated in a 13-month period beginning on September 21, 2006, when the first case appeared, through October 26, 2007, when the last case appeared. In 14 of 16 cases, the species were from the Calliphoridae family. Six from the Sarcophagidae family, one Heleomyzidae (Diptera), and one Coleoptera larvae were found.

Bana and Beyarslan (2012) collected Coleoptera fauna from the surface of a corpse on the Trakya University Güllapoğlu campus in Edirne and conducted a taxonomic study of the samples, which is essential for forensic entomology, that identified 14 insect species belonging to the Coleoptera family.

Another study involved the collection of Diptera fauna, which is important in forensic entomology, on the Güllapoğlu campus of Trakya University in Edirne Province and conducted a taxonomic study, and in another study of the Diptera species in Lüleburgaz district of Kırklareli province, a total of five insect species were identified: Calliphorae vicina and Lucilia sericata from the Calliphoridae family, Muscina stabulans and Musca domestica from the Mucscidae family, and Wolhfahritia magnifica from the Sarcophagidae family (42). Eight insect species were identified (39). In the current study, fly species that are significant in forensic entomology will be identified in the Mustafakemalpaşa district of Bursa, which has not been studied before; therefore, a valuable resource will be created for use in the solution of forensic events for both the country and Bursa province.

MATERIALS AND METHODS

In this study, three Sus scrofa (domestic pigs) were used for the identification of Diptera species that invaded a carcass. The Sus scrofa were provided by the Uludağ University Faculty of Veterinary Medicine. In the experiments carried out in May, July, and October, early on the morning of the first day, the farm pig was anaesthetized with Rampun 2% (Xylazin 400 m/kg) injected by the staff of Uludağ University Animal Hospital at the university's farm, and then euthanized with T61 75mg/kg injection. The euthanized pig was wrapped in nylon bags to prevent contamination and taken to the test area in a cube van. To prevent destruction by

Table 2: Species Identified in the Second Field Study

Determinations	4.07.2017	5.07.2017	6.07.2017	7.07.2017	8.07.2017	9.07.2017	10.07.2017	11.07.2017	12.07.2017	13.07.2017	14.07.2017	15.07.2017	16.07.2017	17.07.2017	18.07.2017
Sarcophaga variegate	+	+	+	+	+	+	+			+					
Sarcophaga lehmanni	+	+	+	+	+	+		+			+	+			
Calliphora vicina	+	+	+	+	+	+	+			+		+			+
Wohlfahrtia magnifica	+	+	+	+	+	+	+				+				+
Lucilia sericata	+	+	+	+	+	+						+			+
Musca domestica	+	+	+	+	+	+								+	
Calliphoridae 3.instar				+	+	+	+	+	+						
Sarcophadigae 3 instar				+	+	+	+	+	+						
Muscidae 3. instar				+	+	+	+	+	+						

predators or scavengers, the pig carcasses were placed in a stainless-steel cage with a 100 cm × 100 cm × 100 cm wire fence at a distance of 10 cm, and the Diptera species collected from them in May, July, and October under different land conditions were examined accordingly. Mature flies and larvae on the rotting Sus scrofa specimens were collected, and seasonal conditions and changes in the species were recorded. The study aimed to identify Diptera species that play a role in determining death-time in forensic studies and to observe their development in varying seasons, temperatures, and humidity values.

The study area is located within the borders of Akçapınar village in the Mustafakemalpaşa district of Bursa Province, 45 km from Mustafakemelpaşa district, 40 km from Nilüfer district, in the northeast side facing the Uluabat lake, the south side facing the village center, where the lake shore consists mostly of reed areas, bushes, and forests of primarily olive and fig trees. It is a region with partially rough terrain. The first test area, which was studied in May, is a grass-covered land area composed of shrubland, facing Uluabat lake on the north side and Akçapınar village to the south, which gets daylight in midday and evening hours, with olive and fig trees surrounding. The second test area, studied in July, is located on the south side of a lake near Uluabat Lake. Its front side faces Akçapınar village and its back side faces Uluabat Lake. It is a soil zone that gets daylight all day and is surrounded by tall bushes and grass. The third test area, studied in October, is a forested area on the south side of Uluabat Lake, to the north of Akçapınar village, and has mostly pine trees on a partial slope and a rugged land structure. Its ground soil structure gets daylight in morning and midday hours.

The sampling process was carried out twice a day, in the forenoon and afternoon, between the dates of May 19 and June 2, 2017, for the first study; between July 4 and 19, 2017, for the second study; and between October 12 and 27, 2017, for the third study. To protect from predators and scavengers, the pigs to be tested were placed in 100 cm × 100 cm × 100 cm stainless steel cages. During collection, a special jumpsuit and gloves were used, and hygiene rules were followed. Physical changes in

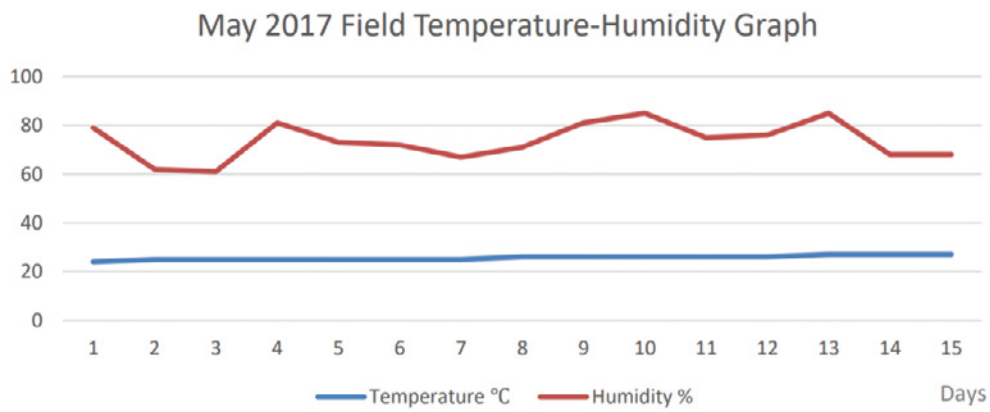

Figure 2: May 2017 First Field Study Temperature-Humidity Graph Jump Suit

the pigs were noted and photographed every day (Fig. 1). Mature insects coming to the carcass were collected with a sweep net, and larvae were collected with special forceps and preserved in 99% alcohol in Eppendorf tubes.

Samples collected in the field studies were brought to the laboratory environment and photographed under a stereomicroscope (Olympus trinocular stereo). Mature insects were pinned on the right side of the scutum region using steel insect needles and turned into museum material. A third of the needle is adjusted to be above the sample and two-thirds below. The collected larvae were stored refrigerated in 99% alcohol in Eppendorf tubes after being photographed. The available literature was consulted to identify the species (1,43–49). This study aimed to identify Diptera species that will play a role in the determination of death-time in future forensic events and to observe their development in different seasons, temperatures, and humidity levels.

RESULTS

The species detected in the study in May, as well as the dates of the larvae reaching the third instar stages, are given in Table 1. The table shows that adults of the Lucilia ampullacea, the Calliphora vicina, and the Muscina stabulans species were seen on the pig carcass on the first two days to the fourth and fifth days of the study, and Lucilia ampullacea and Calliphora vicina were seen on the last day of the study. Also, Muscina stabulans were seen on the 12th day of the study. The table also shows that the third instar stage of larvae belonging to the Calliphoridae and Muscidae families were seen on the sixth day of the study.

In the May study, the average temperature was 25.6°C; the range was from 24°C to 27°C. The average humidity was 73.6%, ranging from 61% to 85%. These data are shown in Figure 2. The average temperature during the period when larvae of the Calliphoridae and Muscidae families passed to the third Instar phases was 24.8°C. Temperature and humidity data in the first field study conducted between May 5 and June 2, 2017, are given in Figure 2.

Species identified in the July study and the dates of larvae reaching the third instar stages are given in Table 2. Mature insects and larvae detected from samples collected in the second field study between July 4 and 18, 2017, are shown in Table 2. Due to high temperature and humidity values during July, Sarcophaga variegata, Sarcophaga lehmani, Calliphora vicina, Wohlfahrtia magnifica, Lucilia sericata, and Musca domestica were found almost every day except on the 12th and 16th days. The table also shows that the third instar stages of the larvae of the Calliphoridae, Sarcophagidae, and Muscidae families were seen from the fourth day of the study.

Table 4 shows that the average temperature In the July study was 30.6°C, ranging from 30°C to

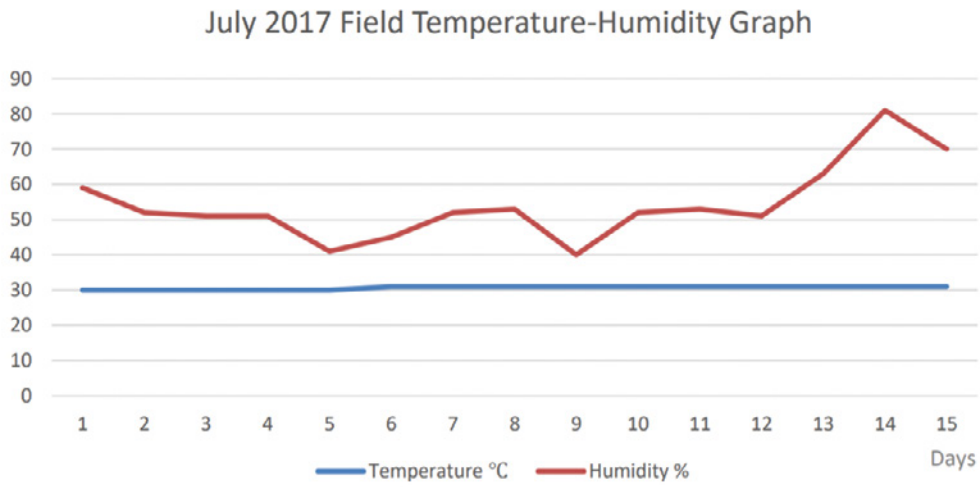

Figure 3: July 2017 Second Field Study Temperature-Humidity Graph

31°C. The average humidity was 54.2% (from 40% to 81%). The average temperature in the period when larvae of the Calliphoridae, Sarcophagidae, and Muscidae families passed to the third instar stages was 30°C. Temperature and humidity data in the second field study (July 4 to 18, 2017) are given in Figure 3.

Species detected in the October study and the dates of larvae reaching the third instar stages are given in Table 5. Mature insects and larvae detected in samples collected in the third field study between October 12 and 26, 2017, are shown in Table 3. The days when Calliphora vicina, Calliphora subalbina, Calliphora uralensis, and Lucilia caesar species were seen are indicated in the table. As shown, the third Instar phases of Calliphoridae larvae were seen on the seventh day of the study.

The average temperature during the October study period, as shown in Figure 4, was 21.2°C (19°C to 23°C). Average humidity was 52.6%, ranging from 29% to 70%. The average temperature in the period when larvae of the Calliphoridae family passed to the third instar stage was 22.2°C. Temperature and humidity data in the third field study, October 12 to 26, 2017, are given in Table 3.

Decomposition rates and morphological changes of the pig carcasses used in the three studies also varied depending on temperature and humidity.

Pig carcass in May study

From the first day, mature insects came and laid their eggs in moist areas, such as eyes, mouth, ears, and anus.

From the second day, swelling of the carcass, especially the abdominal region, was noted.

From the fourth day, dead bruising began in the abdomen and nipple areas, and the number of eggs and larvae increased.

From the fifth day, dead bruising covered the whole carcass.

From the eighth day the carcass entered the decomposition stage.

From the 13th day, the carcass melted, and on the 15th day, it entered the drying phase.

Pig carcass in July study

Due to higher temperatures, dead bruises and swelling began from the first day; the number of eggs and larvae was very high, covering the whole body.

From the second day, the carcass entered the disruption stage, and discharge began in the abdomen and head area.

Melting started on the third day.

On the 15th day, the carcass was completely dried, and its skeletal structure began to emerge.

Pig carcass in October study

Swelling was observed on the second day.

From the third day, dead bruises appeared.

The carcass entered the melting phase on the seventh day.

The carcass entered the drying phase on the 19th day.

DISCUSSION

In three separate studies conducted under varying climatic and field conditions, the decay phases and physical changes of a Sus scrofa (domestic pig) carcass were noted over 15 days in relation to ambient temperature and humidity values, and collected samples were examined in a laboratory. Since flies of the Diptera species are active during the day, samples were collected for each study in the forenoon and afternoon on 15 consecutive days. A total of 12 species were identified—three from the Sarcophadigae family (S. variegata, S. lehmanni, and Wohlfahrtia magnifica), seven from the Calliphoridae family (C. subalbina, C. uralensis, C. vicina, C. vomitoria, Lucilia caesar, Lucilia ampullacea, and Lucilia sericata), and two from the Muscidae family (M. domestica and M. stabulans). In addition, the third instar phases of the larvae examined in the laboratory were determined on a genus basis. As in studies conducted in other parts of the world and in our country, the reason for choosing Sus scrofa (domestic pig) for this study is that their skin structure and digestive system are the most similar to human beings. Forensic entomological studies in Europe and other regions are generally for the purpose of determining the time of death. In contrast, in our country, the aim is to determine the diversity of Diptera species with their geographic distribution. Calliphora vicinia was found to be the most common species in this study, as well as in many studies conducted in our country and elsewhere. Comparing the results obtained in this study with studies conducted in the world and in our country, the following results emerged: Calliphora vicina was the dominant species detected in all three field studies conducted in May, July, and October. Muscina stabulans identified in samples captured in May were detected by Yeşilyurt, Fazlıoğlu, Kubancı, and Kolusayın (2014) in a study in Kırklareli. Musca domestica identified in samples captured in July was also discovered in a study conducted in Samsun and Kırklareli (1,39). Lucilia sericata was detected in the study conducted in Samsun, Edirnem, and Kırklareli, and Wolhfahritia magnifica was detected in the study conducted in Kırklareli. Cal-

Table 3: Species Identified in the Third Field Study

Determinations	12.10.2017	13.10.2017	14.10.2017	15.10.2017	16.10.2017	17.10.2017	18.10.2017	19.10.2017	20.10.2017	21.10.2017	22.10.2017	23.10.2017	24.10.2017	25.10.2017	26.10.2017
Calliphora vicina	+	+		+	+		+					+	+		
Calliphora subalbina	+	+			+						+				
Calliphora uralensis	+			+		+		+					+		+
Calliphora vomitoria	+	+			+	+					+				+
Lucilia caesar	+	+				+						+		+	
Calliphoridae 3.instar							+	+	+	+					

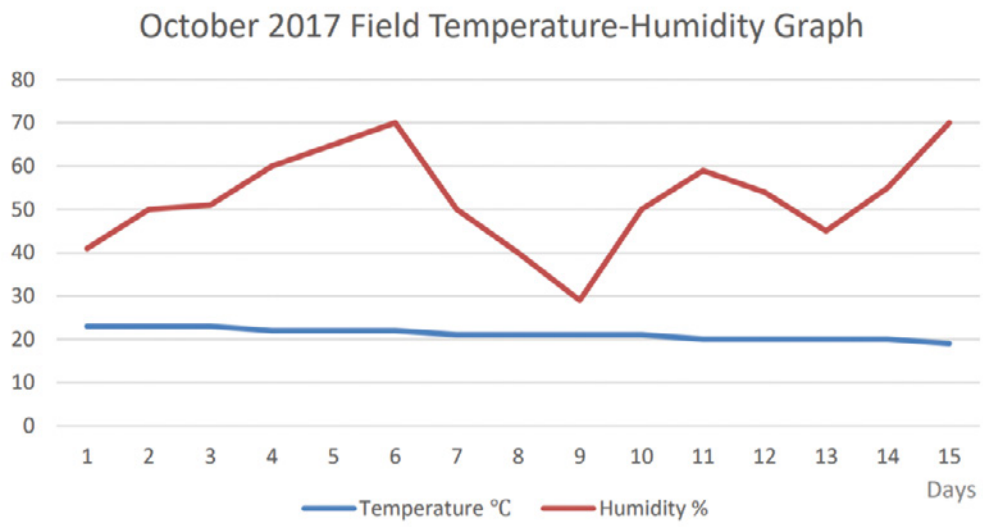

Figure 4: October 2017 Third Field Study Temperature-Humidity Graph

liphora vomitoria, which was identified in samples collected in the October study, was also detected in the study conducted in Edirne. Apart from these, Lucilia ampullaeca collected in May, Sarcophaga variegate and Sarcophaga lehmani collected in July, and Calliphora subalbina, Calliphora uralensis, and Lucilia caesar collected in October in our study were undetected in other studies in the world and in our country.

Temperature and humidity were proven to influence the decaying of the pig carcasses and the development of larvae on the carcass.

- Larvae of the Calliphoridae and Muscidae families were detected in May, when the temperature ranged between 25°C and 30°C and the humidity varied between 60% and 80%. They were seen in the third instar phase beginning on the sixth day.

- Larvae of the Calliphoridae, Sarcophagidae, and Muscidae families were detected in July, in temperatures of 30°C and above and humidity of 40%–80%. The third instar phase began on the fourth day.

- Larvae belonging to the Calliphoridae family were detected in October, in temperatures ranging from 19°C to 23°C and humidity of around 50%. They were seen in the third instar phases beginning on the seventh day.

In line with these results, it is recognized that temperature and humidity are extremely influential in larval development. Higher temperatures and humidity cause an increase in the number of mature insects, which is accompanied by an increase in the number and the development of larvae on the carcass.

To evaluate the varying field conditions where the three studies were conducted, we point out that the first study area is surrounded by olive trees, and since it is a region that receives sun from noon, mature Diptera activities on the carcass were more common in the afternoon. Morphological changes on the pig carcass revealed parallelism accordingly. On seasonal variations in the working range, almost no mature Diptera species were encountered, especially when samples were collected on rainy days. However, the activities of the eggs and larvae on the carcass continued.

The geographical area in which the second study was carried out was very high and brushy. Due to the high average temperature in July and exposure of the pig carcass to the sun from morning hours until sunset, the carcass decayed very quickly. The activities of mature Diptera and larvae continued until the carcass was completely dry.

In the forest area where the third study was conducted, the results are similar to the seasonal values in the first study. It was determined that temperature and humidity affected the rotting stages of the carcass, causing the earliest drying phase to be seen in the July test, when conditions are most intense, and the drying phase began latest in October, when the temperature is lowest. It was seen in these three studies that mature insects invaded the carcass to lay their eggs first in the humid areas of the pig carcass, in line with studies conducted elsewhere in the world and in our country (1). In addition, it was observed that the number of larvae increased due to the decay of the carcass. The number of mature insects decreased especially on rainy days and continued consistently on other days. During this study, it was determined that the major factors affecting the life cycles of Diptera species are air temperature and humidity, in accordance with different geographic structures and daylight periods during the day. High temperature hastened the hatching of the eggs, and the metabolism of the larvae caused the cycle to accelerate. For this reason, knowing all the temperature and humidity values across one year is very important to correctly perform accurate calculations of the post-mortem interval (PMI). If temperature and humidity values are recorded incorrectly, the duration of the development phases will be calculated incorrectly, leading to incorrect PMI calculations. In Bursa province, in order to use entomological data in forensic events, a control group should be trained in the laboratory environment to better observe the developmental stages of the species, and a climate cabinet with a temperature and humidity chamber should be used for this purpose. Thus, mature insects could be obtained by developing Diptera eggs, larvae, and pupae taken from the scene, and the eggs obtained by mating these adults should be developed under conditions similar to the temperature, humidity, and light intensity at the scene. This will facilitate precise calculations of PMI by calculating the development times of Diptera specimens who develop in an environment similar to the conditions at the scene (1,43,45,46,47,48,49,50,51).

Resources should be made available to forensic biology masters programs to adequately train academics in the field of forensic entomology. In large research centers and university forensic medicine departments, a microscope and climate cabinet—the basic equipment of the forensic entomology laboratory—should be available. With these tools, entomological evidence can be more precise in determining species and their age, and they can be useful in education as well as in scientific research. In reviewing other studies conducted in our country, it is clear that they generally used a single geographical location and climate conditions. Unlike other studies, in this study, the rotting stages of pig carcasses were observed under different geographical and climatic conditions, and the diversity of Diptera species that came to the carcasses was observed. Having entomological data available throughout the year for use in judicial events would be much more effective in guiding us in clarifying unsolved events. Likewise, most studies are done in an open field environment. If similar studies were carried out in enclosed conditions in different seasons, the diversity of Diptera on the carcass could be investigated, and data obtained could serve as a resource for additional calculations.

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