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RESEARCH ARTICLE

Effect of the summer and winter seasons on the rate of insect species attracted to the rabbit carcass in Al-Jamʿiyah area / Holy Karbala

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ABSTRACT

Knowledge of the biology and ecology of insect species attracted to corpses is very important in determining the method of killing and the crime scene, and in identifying the species of insects successively occurring on the corpse according to the differences in seasons and regions. This study was conducted in Al-Jam'iyah area in Karbala Governorate for the purpose of identifying the forensically important insect species successively occurring on corpses according to the differences between the summer and winter seasons. The wild rabbit belonging to the order Lagomorpha was used as a vertebrate animal model, where the number of individuals used reached twelve (12) rabbits, with approximate weights ranging between 2–2.5 kg for each animal, with the aim of studying the insect species successively occurring on animal corpses according to the differences between the summer and winter seasons.The study recorded 11 insect species belonging to three main orders, namely Diptera with 6 species, Coleoptera with 5 species, and Hymenoptera. The results showed a clear superiority in the rates of insect attraction to the carcass during the summer season compared with winter, especially in the order Diptera, where the species recorded their highest values in summer, such as Chrysomya albiceps 61.2 and Musca domestica 70, compared with their decrease in winter, 43 and 43.2, respectively. As for the order Coleoptera, the species Dermestes maculatus recorded the highest attraction rate in both seasons, 113 in summer and 102 in winter, whereas Anotylus sp. was the lowest, 0–7.6. As for ants Formicidae, they showed high activity in both seasons, with an increase in winter, 270 compared with 202 in summer. The results showed significant differences at 0.05 for the effect of the seasonal factor, with a clear effect of temperature on flies compared with beetles. These results indicate that seasonal changes directly affect the density and succession of insects on corpses, which enhances their importance in forensic entomology applications, especially in estimating the postmortem interval.

Keywords: *Forensic entomology; insect succession; rabbit carcass; seasonal variation; postmortem interval.*

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INTRODUCTION

Insects are among the oldest living organisms that inhabited the earth. Their appearance dates back to time periods preceding human existence by millions of years. They have shown an exceptional ability to adapt to a wide range of environmental conditions, which enabled them to spread in various terrestrial and aquatic ecosystems. This evolutionary success is attributed to the structural, physiological, and behavioral characteristics they possess, which enabled them to persist and reproduce with high efficiency, in addition to their continuous vital activity and their persistent pursuit of obtaining food resources (Goff, 2009)

Scientific interest in insects of medical, veterinary, and forensic importance also extends to deep historical roots, as the Chinese judge and scholar Song Ci in the thirteenth century is credited with the first systematic documentation of the use of insects in forensic detection, through his accurate observations of the behavior of flies in identifying the crime tool, as mentioned in his book The Washing Away of Wrongs, which is considered the cornerstone in the emergence of this field

With the development of modern sciences, entomology witnessed a qualitative shift represented by the formation of precise specializations, the most prominent of which is forensic entomology, which depends on analyzing the patterns of succession of insects associated with corpses to estimate the time of death. Advances in molecular techniques, especially DNA analysis and gene sequencing, have contributed to bringing about a fundamental transformation in understanding the evolutionary relationships among insects, which enhanced the accuracy of the forensic applications of this science Forensic entomology is defined as an applied branch that employs insects and arthropods in the service of legal investigations. Estimating the minimum postmortem interval (PMI) is considered one of its most important applications, as this is achieved through studying the developmental stages of insects colonizing the corpse. Its role is not limited to this aspect only, but also extends to include detecting the possibility of moving the corpse from one location to another, or identifying the presence of toxic substances or drug effects, in addition to its role in evaluating cases of neglect (Amendt et al., 2011)

Ecological studies have shown that a group of insects, especially in their larval stages, depend in their feeding on decomposed tissues and are known as necrophagous insects, whereas other groups use the corpse as a food resource during different stages of their lives, which reflects the complexity of the food webs associated with the decomposition process (Keiper & Casamatta, 2001; Hobischak & Anderson, 2002)

Systematic analysis of patterns of insect distribution, behavior, and developmental stages at the crime scene provides a rich database. This enables researchers to reconstruct environmental and temporal conditions associated with the incident. This enhances reliability of forensic conclusions (Amendt et al., 2007)

The process of corpse decomposition, as well as the dynamics of succession of insect communities, is subject to the influence of a complex group of environmental and physical factors, including temperature, relative humidity, seasonal changes, and geographical location, in addition to the characteristics of the corpse itself, such as size and the presence of clothing, as well as surrounding environmental factors such as soil type and vegetation cover density, and also the effect of chemicals and drugs (Kyerematen et al., 2013).

With respect to the taxonomic composition of insects associated with carcasses, necrophagous species play a key role. They eat directly through decomposing tissues. In the case of Entrance or Stringing flies related to flies fly might be the foremost colonizers (Anderson, 2013) since they create lunch of and bundle between tastes, respectively.

Scientific literature indicates that the families Calliphoridae, Sarcophagidae, and Muscidae are the most common in association with human and animal remains. They are often the first arrivals at the decomposition site (Chin et al., 2011).

These are followed by other groups including beetles from the families Staphylinidae, Cleridae, Dermestidae, and Silphidae. Ants from the family Formicidae also join, which may affect the remains and contribute to altering the course of the decomposition process (Sikes & Venables,. 2013; Pechal et al., 2014; Byrd & Castner.,2019; Bonacci et al., 2022;). The use of entomological evidence in forensic investigations has gained increasing global attention. This is particularly true in countries of the Northern Hemisphere, where systematic studies have been conducted to develop this field and enhance its practical applications (Williams & Villet, 2006)

MATERIALS AND METHODS

Animals used in the study

The vertebrate animal, the wild rabbit (Lepus sylvaticus(Smith & Johnston 2019–2023 ., Lepus sylvaticus Bachman, 1837), belonging to the order Lagomorpha and the family Leporidae, was used as a vertebrate animal model in this study. The number of individuals used was twelve (12) rabbits, with approximate weights ranging between 2–2.5 kg for each animal, with the aim of tracing the stages of decomposition and studying the insect species successively occurring on animal carcasses.

Slaughtering using a sharp knife to ensure rapid death and reduce the suffering of the animal, while preserving tissue integrity and avoiding excessive mechanical damage that may affect the natural decomposition process or the attraction of insects carried out killing. Immediately after slaughter, the carcasses were handled according to a specific protocol, as they were placed at the field experiment site located in Al-Jam'iyah residential area in Holy Karbala Governorate, to be used in studying insect succession on carcasses under natural environmental conditions. This was followed by field and laboratory study procedures to complete the stages of rearing the larval and pupal stages.

Effect of the seasons of the year on the types of insects attracted to the carcass after its death

The study was conducted during the summer and winter seasons due to the difference in weather conditions in Iraq. Rabbit carcasses were used at a rate of 6 carcasses for each season, and the experiments were carried out in Al-Jam'iyah residential area / center of Holy Karbala. The operations carried out included killing, daily monitoring, collection, preservation, counting, and recording the condition of the carcass and the types of insects attracted until the final stage of carcass decomposition. The samples were later transferred to the laboratory for counting and diagnosis purposes. The study adopted the interaction of the summer and winter seasons within the research area and the relationship of these variables with the types of insects attracted to the rabbit carcass, as the same experiment was conducted with the same replications in the two mentioned seasons.

Laboratory study (Laboratory Experiments)

The laboratory study was conducted with the aim of obtaining the adult stages of the dominant species of flies, especially metallic flies, which were collected from the carcasses used in the field study. The study also aimed to confirm the morphological diagnosis of the species obtained through the daily collection of insects during the experiments.

The larvae were collected manually using direct collection methods from the animal carcasses on which they were found. Then, the samples were divided into two groups to be reared laboratory under controlled conditions:

First group:

The larvae were placed in open plastic containers covered from the top with gauze tops. Each box contained a rabbit head as a food source for the larvae, and a few drops of water were added to maintain the moisture of the food medium. This rearing method was based on the study of (Gruszka et al., 2021) which used plastic containers provided with gauze mesh covers to provide suitable conditions for rearing larvae.

Second group:

The larvae were placed in an amount of moist soil suitable for their number, and the soil was supplied with a 10% sugar solution to maintain moisture and provide an additional source of energy. The samples were left under laboratory conditions until they reached the pupation stage, which took on average between 5–7 days depending on the fly species. After that, the pupae were transferred to containers containing suitable amounts of sand to provide an appropriate medium for completing the pupal stage and transforming into the adult insect, during a period ranging between 12–17 days depending on the differences among species.

Collection and preservation of adult stages

After the completion of transformation and the emergence of adult insects, the samples were carefully collected and placed in the refrigerator for 3.5 minutes at a temperature of 3°C for the purpose of inhibiting their movement and facilitating their preservation. The specimens were preserved, and specialized international taxonomic keys were adopted, including (Whitworth, 2006; Mazur, 2011; Háva, 2003) for diagnosing the different insect species, with the assistance of forensic entomology references (Byrd & Castner, 2010) in order to ensure the accuracy of diagnosis according to the standard morphological characters of each taxonomic group.

RESULTS AND DISCUSSION

The results of Table (1) showed the presence of different species that were diagnosed and belong to families and insect orders of forensic importance. The number of species diagnosed at the level of the order Diptera reached six species belonging to three families, namely Calliphora vicina, Chrysomya albiceps, and Lucilia sericata, which belong to the family Calliphoridae; the two species Sarcophaga africa and Sarcophaga vagans, which belong to the family Sarcophagidae; and the species Musca domestica, which belongs to the family Muscidae. The study of (Szpila et al., 2023) in the journal Insects indicated that species such as Calliphora vicina and Lucilia sericata are among the first colonizers under temperate and subtropical conditions, and models of their larval growth are widely used in forensic estimations.

As for the level of the order Coleoptera, five species were diagnosed, belonging to four families, namely the species Saprinus subnitescens from the family Histeridae, the two species Dermestes maculatus and Dermestes sp. from the family Dermestidae, the species Necrobia rufipes from the family Cleridae, and the species Anotylus sp. from the family Staphylinidae. The study of (Velásquez & Viloria, 2021) in Journal of Medical Entomology confirms the successive sequence, succession, of insects on carrion, where beetles arrive after flies by a period of time. Some species belonging to the order Histeridae arrive during the active decay stage for predation, whereas Dermestidae appear clearly in the dry stage to consume dry tissues and skin. From the above, it becomes clear that beetles, compared with flies, may reflect a less severe response to sharp seasonal changes, or this may be due to their diversity.Whereas at the level of the order Hymenoptera, different species of ants belonging to the family Formicidae were found. The appearance of ants in large numbers, whatever the case may be, may be predatory on larvae and eggs, which may sometimes disrupt the process of natural succession of insects.

The table also shows a clear variation in the rate of attraction of insect species belonging to the three orders according to the difference between the summer and winter seasons. In the summer season, the highest attraction rate at the level of the order Diptera was recorded for the fly Chrysomya albiceps, and its number rate reached 61.2 insects, whereas the lowest attraction rate was 16.3 insects for the fly *Sarcophaga vagans*. As for the level of the order Coleoptera, the highest attraction rate appeared for the beetle *Dermestes maculatus*, and its number rate reached 113 insects, while the lowest attraction was for the beetle Anotylus sp., which reached 7.6 insects. At the level of the order Hymenoptera, it appeared that the highest attraction rate occurred in the family Formicidae, which includes different species, and its number rate reached 202 insects.

As for the winter season, Table (1) showed that the highest attraction rate at the level of the order Diptera appeared in Musca domestica and reached 43.2 insects, and the lowest occurred in the species Sarcophaga vagans and reached 12.1 insects. This was contrary to what occurred in the summer season. The same thing occurred at the level of the order Coleoptera in terms of the highest and lowest attracted species, where the species Dermestes maculatus recorded the highest attraction rate and reached 102 insects, and the lowest appeared in the species Anotylus sp. and reached 0. At the level of the order Hymenoptera, ants of the family Formicidae, with their different species, recorded the highest attraction rate compared with the summer season, 270 insects in winter compared with 202 insects in summer. Based on the foregoing, it becomes clear that the different seasons of the year, such as summer and winter, have a major effect on the attraction of insect species to any area; that is, the diversity and difference of insect succession on the corpse after death are greatly affected by the seasons. Differences in attraction rates were observed for all diagnosed species according to the season, and high differences were observed in their presence rates between the winter and summer seasons. All species recorded in this study gave the highest presence in the summer season compared with the winter season, in addition to the presence of significant differences at the 0.05 level for the interaction between the two seasons and the rate of species numbers at the level of the order Diptera.

The rate of the numbers of species attracted to the rabbit carcass during the winter season reached, respectively: 34.2, 43, 32, 31, 12.1, and 43.2 insects for the species *Calliphora*

Table (1) The effect of the density and types of insects attracted to a rabbit carcass in the Al-Jam'iya area, Al-Maqdisa Governorate.

Winter From 6 December to 14 January			The number and types of insects attracted, by season		LSD for interaction between preparation rate and species at the rank level
Orders	Families	Types	Winter From 6 December to 14January	Summer Term From 7June to 24 June	
Diptera	Calliphoridae	Calliphora vicina	34.2	57.3	12.47
		Chrysomaya albiceps	43	61.2	
		Lucilia sericata	32	45.3	
	Sarcophagidae	Sarcophaga Africa	31	56.2	
		Sarcophaga vagans	12.1	16.3	
	Muscidae	Musca domestica	43.2	70	
LSD			8.82	5.09	
Coleoptera	Histiridae	Saprinus subnitescens	21	43	5.63
	Dermistidae	Dermestes maculates	102	113	
		Dermestes sp.	2	32	
	Cleridae	Necrobia rufipes	34	54.2	
	Staphylinidae	Anotylus sp.	0	7.6	
LSD			3.98	2.52	
Hymenoptera	Formicidae	Sp.	202	270	25.83
LSD			20	24	

vicina, *Chrysomya albiceps*, *Sarcophaga africa*, *Lucilia sericata*, *Sarcophaga vagans*, and *Musca domestica*, respectively, compared with 57.3, 61.2, 45.3, 16.3, 56.2, and 70 insects in the summer season, respectively.

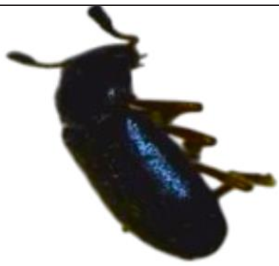
As for the level of the order Coleoptera, the rate of the numbers of species attracted to the rabbit carcass during winter reached, respectively, 21, 102, 2, 34, and 0 insects for the species Saprinus subnitescens, Dermestes maculatus, Dermestes sp., Necrobia rufipes, and Anotylus sp., respectively, compared with 43, 113, 32, 54.2, and 7.6 insects in summer, respectively.

As for the level of the order Hymenoptera, the rate of the numbers of species belonging to the family Formicidae in winter reached 202 insects and in summer 270 insects.

Based on the study of (Montaldo et al., 2022) and other studies, it becomes clear that in summer, with the rise in temperatures, the colonization activity of insects increases and their life cycles accelerate, which leads to greater abundance and higher numbers, especially for flies. Whereas in winter, abundance and diversity decrease noticeably, and the group may be limited to specialized species that tolerate cold, such as Calliphora vicina, which is known for its activity at low temperatures.

The study of (Montaldo et al., 2022) in Forensic Science International also found that the structure of the insect community on corpses differs greatly between warm and cold seasons, not only in number but also in the sequence of arrival of species, which confirms the importance of seasonal differences in forensic applications. This insect diversity and the large difference in rates between one species and another during the same period are attributed to the difference in seasons, as some insects prefer summer and spring more than other seasons, and some of them may tend, in close or low percentages, to one of these two seasons, summer or spring. This agrees with

Table (2) shows the insect species recorded during the study

 *Calliphora vicina*	 *Chrysomya albiceps*
 *Sarcophaga vagans*	 *Musca domestica*
 *Saprinus subnitescens*	 *Sarcophaga africa*
 *Necrobia rufipes*	 *Dermestes maculatus*
 *Anotylus. Sp*	

the results of the study conducted in South Africa by (Kelly et al., 2008) where they found that seasons had a major effect on the composition and diversity of flies, especially flies of the family Calliphoridae, and did not greatly affect the presence, attraction, and diversity of insects of the order Coleoptera.

Whereas some research indicated that some forensic insects are highly associated with seasons and regions; that is, some of them prefer cold weather, but they do not follow this behavior in other regions (Wang., et al 2020) The results of the current study did not agree with the results of the study conducted in Brazil by (Shaurub, et al ., 2024) where they found that the seasons of the year had no effect on insect diversity and numbers, and that most fly species are greatly affected by place and environmental location with regard to their attraction to corpses. Accordingly, the difference between the results of the current study area and some related studies may be due to the difference in the study area and the climatic conditions within the summer and winter seasons.

Statistical Results:

- The least significant difference (L.S.D) value at the 0.05 level for the interaction between the summer and winter seasons and the rate of species and numbers of insects of the order Diptera is 12.47.
- The least significant difference (L.S.D) value at the 0.05 level for the interaction between the summer and winter seasons and the rate of species and numbers of insects of the order Coleoptera is 5.63.
- The least significant difference (L.S.D) value at the 0.05 level for the interaction between the summer and winter seasons and the rate of species and numbers of insects of the order Hymenoptera is 25.83.

CONCLUSION

The study concluded that seasonal variation has a clear effect on the abundance and succession of insects attracted to rabbit carcasses in Al-Jam'iyah area, Holy Karbala. Insect activity was generally higher during the summer season, especially among Diptera species, due to the effect of higher temperatures on insect attraction and development. Coleoptera species also showed seasonal differences, while ants of the family Formicidae remained active in both seasons. These findings confirm the importance of considering seasonal conditions when using insect evidence in forensic entomology, particularly for estimating the postmortem interval.

Statistical results

- The least significant difference (LSD) value at the 0.05 level for the interaction between the summer and winter seasons and the species and abundance of Diptera insects is 12.47.
- The least significant difference (L.S.D.) value at the 0.05 level for the overlap between the summer and winter seasons and the species richness and abundance of Coleoptera insects is 5.63.
- The least significant difference (L.S.D.) value at the 0.05 level for the overlap between the summer and winter seasons and the ratio of species and numbers of insects of the order Hymenoptera is 25.83

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