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Insect succession and carcass decomposition during spring and summer in An-Najaf province-Iraq.

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Abstract

Forensic entomology is the application and study of insect biology to medico-legal matters. This study was conducted to gather database information about forensically important insects and their succession on decomposing exposed rabbit carcasses at rural area of An-Najaf province-Iraq during two seasons spring and summer of year 2014. Ten species from 7 families under 3 orders were successfully identified during the study period, these orders were Diptera, Coleoptera and Hymenoptera. The most important families of Diptera to breed on carcass were Calliphoridae such as *Calliphora vicina*, *Chrysomya megacephala*, *Chrysomya albiceps*, *Lucilia sericata*, and Sarcophagidae such as *Sarcophaga africa*. Dermestidae was most important families of Coleoptera such as *Dermestes maculatus*. All of these species can used as forensic indicators to estimate the PMI. The majority of the collected species were abundant during Spring than Summer. The most important species with respect to abundance and frequency were *C. vicina*, *Chrysomya albiceps* and *Sarcophaga africa*. Four stages of decomposition were observed (fresh, bloated, decay and dry stage). The duration of it varies with temperature and relative humidity of each season. The total period of decomposition of carcasses was 21 days in Spring, while reached 12 days in Summer. There was interaction between decomposition stages of carcasses and colonization by insects species arriving to the carcass. The succession patterns of insect on exposed carcasses occurred in a predictable sequence that varied across seasons. Carcasses placed in spring attracted a more diverse assemblage of insects than Summer-placed carcass. Members of Calliphoridae-*Calliphora vicina* were the first dipterans colonizers and breed on a carcass in Spring, While the *Chrysomya albiceps* was the first colonizer during Summer. The second wave of insects were members of Coleoptera: *Dermestes maculatus*. Adults of *Cataglyphis sp.* continued in appeared during two seasons from the advanced stage to the end of later stages..

Keywords: Insect succession, Decomposition

INTRODUCTION

Knowledge of the distribution, biology and behavior of insects found at a crime scene and the environmental factors can provide an information on where, when and how the crime was committed (1).

Four or five stages of decomposition were observed in carcasses: fresh, bloated, decay, decay-adipocere and dry (2), but each stage of decomposition is attractive to a different groups of necrophagous insects. Some are attracted directly by the carcass, which is used as a food source or an oviposition medium, whereas other species are attracted by the large aggregation of other insects, which provide them a food resource (3). Carrion decomposition and succession patterns of insect are influenced by many factors e.g. geographic location, habitat, season, temperature, humidity, insect abundance and carrion accessibility. Furthermore, many of these factors are inter-related and even the decomposition rate and insect succession are inter-dependent. (4,5). The using insects in death investigations belong to several reasons: 1) insects are usually the first to find a decomposing corpse. 2) the arthropod fauna on a corpse changes in a successional sequence as modifications of the carcass occur (decomposition stages). 3) The fauna on a corpse is often ignored when investigators examine the death scene (6).

In spite of the since the 19th century entomology has been used in forensic applications (7), in some parts of the world research on this topic has been neglected. While most northern hemisphere countries have acknowledged entomological evidence, many southern hemisphere countries appear to be lagging in this regard. This is partly because entomological research in these countries has not been of an explicitly forensic application until recently (8). The importance of the subject at the present time due to the severe current events to the country whereas increased in recent years murder scene, terrorism and suicide. The aim of this study to determine the insect succession pattern to forensically insects on the exposed rabbit carcasses in rural sites during two seasons. This study consider one of the few studies in Iraq and the first in An-Najaf province.

MATERIAL AND METHODS

Study sites

The province of An-Najaf is one of the most important cities in Iraq. Locate to the Southwest area of the country (9). This study was conducted during summer and spring of 2014 at rural site in An-Najaf province. Al-Quzwiniya region represented the site of study (32° 6,44N 44°22,6E) which was agricultural location outside the city with clay soil and it is surrounded by palm trees from two sides and fields of wheat and other type of plant. Near the site situate cattle and sheep farms. The site of the experiments was selected on the basis of specific criteria. It was also necessary to experiment sites are isolated enough to reduce human interferences and scavenger disturbances.

Experimental Animals and Cages

In this study, used six of the mature rabbits ranging in weight from 1.5-2 (± 0.5) Kg. and different in colors. It had been purchased locally from a local market and used as models instead of human corpse. They were slaughtered by knife in the study sites. At each season, three cages were used. The rabbits carcasses were protected from scavengers by a metal cage (60 X 60 X 60) cm³. which was specifically designed to allow for the arrival of insects and prevent them from leaving. Each cage consisted of two layers, the outer layer was a network of rigid steel to prevent the predators and allow the insects to reach. The inner layer was the network fabric tulle with small enough cracks to prevent insects from escaping. The openings were made on two sides of the cage to facilitate accessing and taking out the rabbit carcass (10).

Experimental design and Sampling:

Three rabbits were used in each season. Through experiment day inspection of the carcasses was done every day during early decomposition stages from 9 Am to 12 P.m. then, every two days in later stages. The rabbit carcasses were distributed in the three cages. The distance between these cages was 50 m. Sticky traps were placed on after half a meter of each cage. Date of killing and number of carcasses were determined for each cage. Flying insects were captured by an aerial net as well as the insects in the adhesive traps, all

were removed and assembled in labeling plastic containers and transported to the laboratory for identification and counting. Larval specimens were collected randomly using gentle forceps or spoon, and directly placed in numbered and dated vials and transporting to the laboratory for further identification and counting (11). Beetles were collected by hand, and then killed by ethyl acetate and placed in numbered and dated vials containing 70% alcohol until come back to the laboratory for further identification (12). This sampling design allowed collection of information on different species of insect succession and their composition on the rabbit carcasses at different stages of decomposition; in addition, ambient temperatures, relative humidity, and duration of each stage in both seasons were recorded.

In the laboratory the collected larvae were divided into two groups: the first group was killed in near-boiling water to avoid shrinking them, then placed in vials containing 70% alcohol for further identification (13). The second group was kept alive and reared in the laboratory to obtain the adults for sure species identification, by transferring them into dry glass jar with small amounts of minced chicken liver or beef lung on a layer of Sawdust to allow larvae to be pupate (14). Daily, they were observed and moistened with water. The collected flying insects and beetles were killed by freezing and mounted on insect pins. The specimens were observed under dissecting microscope and identified to order, family and species level according to specific keys (15,16). To confirmation, some samples were sent to the Natural History Museum/University of Baghdad, Iraq.

RESULT

A total of 1066 individuals were collected during this study, the order of Diptera comprised (854) ind. 80.11% and the order of Coleoptera and Hymenoptera comprised (109, 102) ind. (9.66, 10.23%) respectively (Table 1).

Four stage of decomposition were observed during this study (fresh, bloated, decay and dry stage) which were recognized visually by morphological characters (figure1). The duration of it varied with temperature and relative humidity of each season the total period of decomposition of carcasses was 21 days in Spring, while reached 12 days in Summer (Table 2). The four stages of decomposition described as below:

1-Fresh stage: This stage begins at the moment when the corpse were excised and installed in the experiment box and continues until bloating is first evident. This stage characterized by that there is no morphological changes nor odor of decay. This stage lasted longer period in Spring (3) days, while the lowest period was in Summer (1) day.

2-Bloat stage: It begins with simple swelling of the samples, odor of decay became noticeable. A tiny amount of decomposition fluids were seep under the specimens. Longer period was (4) days in Spring, in contrast (3) days in Summer.

3-Decay stage: This stage was characterized by release of gases and deflation of samples. This stage lasted longer period in Spring (6) days at rural site, and lowest period (4) days in Summer season.

4- Dry stage: At this stage most of the fleshy tissue disappeared, odor started to fade and the specimens consist of only dry skin and bone. This stage lasted longer period in Spring (8) days and lowest period (4) days in Summer.

Succession pattern of insects during Spring

Figure (1) showed the insect succession of forensically important insects on the carcasses during Spring season. Larvae and adults of Diptera as well as Adults of Coleoptera and Formicidae were observed on the carcass throughout the experimental time. Six dipteran species were observed during this experiment (*Musca domestica*, *Calliphora vicina*, *Chrysomya megacephala*, *Chrysomya albiceps*, *Lucilia sericata*, *Sarcophaga africa*). In addition three species belong to the order Coleoptera *Dermestes maculatus*, *Saprinus sp.* and *N. rufipes* and one species of ant *Cataglyphis sp.* which belong to Formicidae family. **In the fresh stage** flies appeared from the first day as adults and continued their development in all decomposition stages of the rabbit carcasses, the first arrival to the rabbit carcasses were *C. vicina* and *M. domestica* during the 1st day and collected as adults. **In the bloated stage**, calliphorid flies, sarcophagid flies waves were noticed as adults and immatures at 3rd day, while, muscid flies wave were noticed as adults. In addition, the adults of *Cataglyphis sp.* *C. megacephala* observed more abundant during 4th-7th day. Also, the abundance of *C. vicina*, *Ch. albiceps*, *L. sericata*, *M. domestica* and *S. africa* increased during 6th and 7th day. The adults of *Cataglyphis sp.* appeared

during this stage. **In the decay stage**, when a strong odor was noted, the abundance of the breeders from family Calliphoridae and Sarcophagidae increased especially during the first two days of this stage. Second wave of insects begin to visit the rabbit carcasses, adults of *D. maculatus*, *Saprinus sp.* and *N. rufipes* were observed. The adults of *Cataglyphis sp.* continued in appeared during this stage. **In dry stage**, the flies begin to decrease and left the carcasses when the odor disappeared and the nutrient matter became very little. Immatures of Calliphoridae and Sarcophagidae and adults of Muscidae also, were collected. *C. vicina* was collected as adults .In addition, *D. maculatus*, *Saprinus sp.* and *N. rufipes* were continued to appear during this stage to be the last appearance at 21th day .As for the *Cataglyphis sp.* it was viewed during the first three days of this stage .

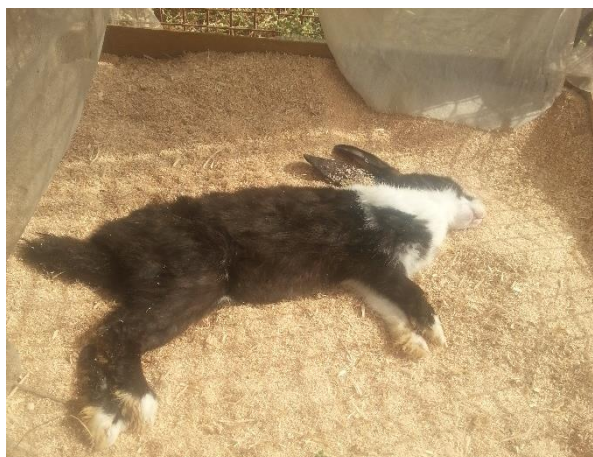
Succession pattern of insects during Summer

Figure (2) showed the insect succession pattern on the carcasses during Summer season. Larvae and adults of Diptera as well as Coleoptera and Formicidae adults were observed on the corpse throughout the experimental time. four dipteran species were observed (*M. domestica*, *Ch. Megacephala*, *Ch.albiceps* and *S.africa*). The same previous three species belong to the order Coleoptera *D. maculatus*, *Saprinus sp.* and *N. rufipes* in addition the ants of *Cataglyphis sp.* **In the fresh stage**, the duration of this stage was very short. First arrival to the rabbit carcasses were adults of *Ch.albiceps* and *M. domestica* . *Cataglyphis sp.* exists as a low rate .**In the bloated stage**, adults of *Ch.albiceps* and *Ch. megacephala* waves were noticed at 2nd day and collected as adults and immatures at 3rd day. *Ch. Megacephala* was most abundant during this stage.While, *M.domestica* adults and adults and immatures of *S. africa* continued to appear .The three species of beetles *D. maculatus*, *Saprinus sp.* and *N.rufipes* were started to appear at the end of this stage. Either, the ants *Cataglyphis sp.* continued to appear in large numbers. **In the decay stage**, during the beginning of this stage when a strong odor was noted, the abundance of the immatures of flies increased especially *Ch.albiceps* and *S. africa*. Either beetles of *D.maculatus*, *Saprinus sp.* and *N. rufipes* were continued to visit the rabbit carcasses. **In dry stage**, the waves of flies started to decrease and beginning left the rabbit carcasses except immatures of *S. africa* .While, adults of beetles continued to appear during this stage. As for the *Cataglyphis sp.* , it was appeared during this stage during 8th-10th day.

Order	Spring	Summer	No. of individual	% individual
Diptera	535	319	854	80.11
Coleoptera	55	48	103	9.66
Hymenoptera	47	62	109	10.23
Total	637	429	1066	100

Table (1): Taxonomic composition of the insects species on exposed rabbit carcasses in An- Najaf province during study period, 2014

A: Fresh Stage



B: Bloat Stag





B: Decay Stage



C: Dry decay

Figure (1): Stage of Decomposition on Exposed rabbit carcasses
A-Fresh stage B-Bloat stage C-Decay stage D-Dry stage

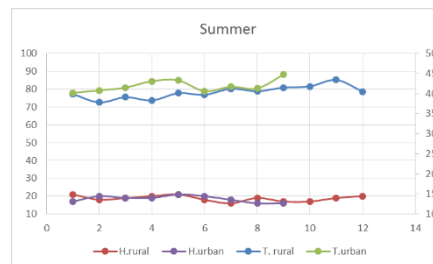
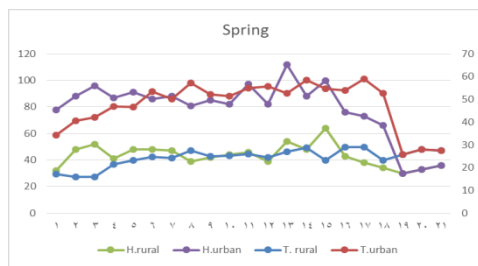


Figure (2):Ambient Temperature (°C) and Relative Humidity in exposed Rabbit during study at two site.
(A) Spring (B) Summer

Table (2) Decomposition stages and its Durations for the Rabbit Carcasses at Rural Site During Study Period

Season	Average T. °C	Stages of Decomposition(day)				Total period(day)
		Fresh	Bloat	Decay	Dry	
Spring	25.13	3	4	6	8	21
Summer	38.45	1	3	4	4	12

Figure (1): Succession pattern of insects on exposed rabbit carcasses in Spring at rural site during study period

Insects Taxa			Stage of Decomposition (days)																
Order	Family	Species	Fresh			Bloated				Decay					Dry				
			1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22
Diptera	Calliphoridae	<i>Calliphora vicina</i>	—			—				—									
		<i>Chrysomya albiceps</i>				—				—									
		<i>Ch. megacephala</i>				—				—									
		<i>Lucilia sericata</i>				—				—									
	Muscidae	<i>Musca domestica</i>	—			—				—									
	Sarcophagidae	<i>Sarcophaga africa</i>				—				—									
Coleoptera	Dermestidae	<i>Dermestes maculatus</i>								—					—				
	Histeridae	<i>Saprinus semistriatus</i>								—					—				
	Cleridae	<i>Necrobia rufipes</i>								—					—				
Hymenoptera	Formicidae	<i>Cataglyphis sp.</i>				—				—									

- It represents a very small number of insects
- It represents a small number of insects
- It represents a moderate numbers of insects
- It represents a a large number of insects

Insects Taxa			Stage of Decomposition (days)											
Order	Family	Species	Fresh	Bloated		Decay				Dry				
			1	2	3	4	5	6	7	8	10	12	14	16
Diptera	Calliphoridae	<i>Calliphora vicina</i>												
		<i>Chrysomya albiceps</i>	—	—	—	—	—	—	—	—				
		<i>Ch. megacephala</i>		—	—	—	—	—	—	—				
		<i>Lucilia sericata</i>												
	Muscidae	<i>Musca domestica</i>	—	—	—	—	—	—	—	—	—			
	Sarcophagidae	<i>Sarcophaga africa</i>		—	—	—	—	—	—	—	—	—	—	—
Coleoptera	Dermestidae	<i>Dermestes maculates</i>				—	—	—	—	—	—	—	—	—
	Histeridae	<i>Saprinus sp</i>				—	—	—	—	—	—	—	—	—
	Cleridae	<i>Necrobia rufipes</i>				—	—	—	—	—	—	—	—	—
Hymenoptera	Formicidae	<i>Cataglyphis Sp</i>	—	—	—	—	—	—	—	—	—	—	—	

Figure (2): Succession pattern of insects on exposed rabbit carcasses in Summer at rural site during study period

- It represents a very small number of insects
- It represents a small number of insects
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- It represents a large number of insects

DISCUSSION

This study was conducted on forensically important insects that were noticed on decomposing exposed rabbit carcasses at rural sites during two seasons Spring and Summer of the year 2014 in An-Najaf province. To study carcasses ecology and insect activity, it was used the rabbits as animal models in this study as many researchers in different geographic locations and areas (17,18). The results obtained from this study provided other information to forensic entomologists by diagnosing Diptera, Coleoptera and Hymenoptera species that are associated with rabbit carcasses in An- Najaf province-Iraq at which no researches on forensic entomology have been performed before. Field observations supported by the data recorded in the present

study showed that all collected insect species were more abundant during the moderate temperatures of Spring than during hot Summer seasons (Figure1). These data correspond to the previous studies carried out by (19) which founded the species composition are also influenced by season factors and carcass microenvironment. The decomposition is a continuous process, in spite of this, in the present study, it was evident the decomposition process in the form of four stages (fresh, bloated, decay and dry). These observations were also defined by many researchers (20,21,22,23). The mean ambient temperatures during spring and summer were 25.13°C and 38.45°C, the total duration of decomposition reached to 22, 14 day respectively. That may due to the low temperatures associated with low insect activity. The critical factor in the determination of the decay rate was ambient temperature in various seasons (24, 17). The activity of forensically important insects arrival at carcasses and development were the important factors contributing to the decomposition of a carcass. (25) In addition, we could assume that the high temperature in Summer in the current study had accelerated the decomposition process and it means that the carcasses are reduced to bones in a shorter period leading to reduction of insects colonization time (26,27, 10) . The families of insects were present on carcasses in both seasons spring and summer, but with different seasonal faunal composition and played an important role in the decomposition of rabbit carcasses. Only the families Calliphoridae, Sarcophagidae and Muscidae belong to the Diptera (28) and the families Dermestidae, Cleridae, Silphidae and Histeridae (29,30) belong to the Coleoptera are the most important to be used in forensic entomology. These species of insects don't visit carcass at the same time but in 'sequential waves' (31). In general, in this study the succession patterns were similar to those described by (28) and (32). In the fresh stage, there is no odor or visual effect obvious to man, however, few calliphorid and sarcophagids flies are able to arrive at early decomposition stages (33). Fly colonization was in high peak in bloated and decay stages whereas the odor become evident and the carcasses has been occupied by larvae and eggs (34). The flies deposit eggs on a carcass after putrefactive odors dispersed and prefer oviposit in areas were colonized by larvae of Calliphoridae (35). The initial colonizers of the carcasses were the adults of the Calliphoridae and Muscidae families and then arriving Sarcophagidae adults (36,37). In succession pattern on carcass, the primary species were *C. vicina* and *L.sericata* (calliphorinae), whereas *Ch. albiceps* (chrysomyinae) was a secondary species. This occurs wherever the members of subfamilies are sympatric (17), a flies of chrysomyinae can act as primary species in areas where flies of calliphorinae are absent (38) . While, It was reported even when calliphorinae is also available, the members of Chrysomyinae can represent as primary flies (39). In this study, *C.vicina* was well represented in spring but no during Summer, indicative of a preference for cooler temperatures (40). *M.domestica* collected as adults and no larvae visited carcasses that they indicate it was visited carcasses to feed not to breed. However, The same results were recorded by (41) and (32). It was found that the *Ch. albiceps* is an aggressive predator feeding on other larvae of dipterous present on carcasses as well as decaying tissues (17,42). This interpret the occurrence of other fly species larvae even Calliphoridae relatively in fewer numbers when they found with the *Ch. albiceps* on the same carcass. Generally it has believed that flies of Chrysomyinae represent primary species in absence subfamily of calliphorinae (21). In this study it was appeared that the *S. africa* were an important components of carcass insects. However, some studies mentioned that in tropical regions and warmer temperate the flesh flies are primary colonizer of carcasses (43).While , in other studies it was considered secondary species in cooler regions (24). During this study, three families belonging to the order of Coleoptera were collected Dermestidae, Histeridae and Cleridae beetles were collected. In two seasons, a distinct beetle fauna was available and it played an important role in the process of tissue-clearing. In general, they were more important during later stages of decomposition, as has been stated by the earlier researchers (44 ;39). Dermestidae continued to presence until the dry stage (29) as also observed in other studies by (23) and (45).

The third category contains the omnivorous species, ants *Cataglyphis sp.* that belongs to the Formicidae family, which fed on both carcasses and arthropods associated with it and appeared thought year except Winter. Accordingly may have some role in the decomposition process and have forensic importance. (39) observed ants to feed on moist areas around the eyes, nose, mouth, and anal region during the fresh stage and on dead flies, dead larvae, skin of carrion, etc. and on maggots and pupae during the later stages of decay. (25) point out that ants can cause an interpretation problem relating to time since death. Ants may carry away eggs and the population of the next generation of colonizers may be reduced.

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