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Insect Succession and Decomposition of Buried Rabbits During Two Seasons in Al Kufa City -Iraq.

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ABSTRACT

Forensic entomology is the application and study of insect biology to criminal matters. This study was conducted to gather database information about forensically important insects and their succession and decomposition on decomposing buried carcasses at two depths in Al- Kufa city - Iraq during Winter and Summer season during 2015, usage rabbits as experimental models. Five species were successfully identified from study sites *Chrysomya albiceps*, *Sarcophaga africa* (Calliphoridae) *Dermestes maculatus* (Dermestidae), *Saprinus sp.*(Histeridae) and *Cataglyphis* sp.(Formicidae) .All of these species can used as forensic indicators to estimate the PMI. Four stages of decomposition were observed(fresh, bloated, decay and dry) at two depths 20 and 40 cm. Temperature and depth of buried influenced the stages of carcass decay and insect activity and abundance .The total duration of carcass decomposition at depth 20 cm was faster than that at 40 cm also in Summer compared with Winter seasons . Succession pattern of insect on buried carcasses varied across two seasons. Larvae of *Ch.albiceps* at depth 20 cm were collected in most abundant with other species. Coleopteran species were observed at the end of the bloated stage at depth 20 and 40 cm. These results show that these insects are important in carcass decay and are, therefore of forensic importance.

Keywords: insect, rabbits, decomposition.

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INTRODUCTION

"Forensic entomology is the study of arthropods, including insects in a legal context" (1).It can be divided into three main fields: urban entomology, stored products entomology and medico-legal entomology(2).Medico-legal entomology is a tool that is frequently used to estimate the time interval between death and the discovery of the corpse, known as the post mortem interval or PMI (3).However, the distribution study, behavior and biology of insects found at the scene of crime and the environmental factors can finding an information on how ,where and when the crime was occurred(4). Some valuable information can provide by forensic entomologists such as estimating of time since death (PMI) as well as other valuable information concerning the circumstances surrounding the corpse including season and location of death, movement or storage of remains after death, specific sites of injury on the corpse, use of drugs, and even more in linking a suspect to the scene of a crime, in child neglect and sexual molestation. (5).

There are more than sixty families of insects which play an essential role in the carcass ecology(3). Only the families (Calliphoridae, Sarcophagidae and Muscidae) of Diptera and the families (Silphidae, Staphylinidae, Cleridae and Dermestidae) of Coleoptera are the most important to be used in forensic entomology (6 , 7). (5) studied various factors influencing decomposition as well as succession patterns of insects. They observed also that in bodies underwater and in burial, process of decomposition was delayed. Even in burial conditions, cold climate combined with the type of soil effectively inhibited the decomposition process and accessibility of buried pig carcass to insects (8). Decomposition of carcasses in buried is affected by the elimination of major macroclimatic effects, but limits the timing of arrival of carrion insects. Although, many of the insects associated with exposed carcasses are restricted from colonizing a buried carcass (9). The aim of this study to determine the forensic insect succession pattern on buried rabbit carcasses at the urban locations during winter and summer seasons.

MATERIALS AND METHODS

Study sites

This study was conducted in Al Kufa city in Iraq within the campus of the University of Kufa (32°, 2,23N 44°22,6E) from the east center of An-Najaf province , about 170 km south of Baghdad, and 10 km northeast of An –Najaf province. It is located on the banks of the Euphrates River. The estimated population in 2003 was 110,000 (10).

Experimental Animals and Cages

This experiment was conducted during winter and summer season of 2015. Two depths 20 and 40 cm were selected to bury the carcass under the surface of the soil. Twelve rabbit carcasses were used six in each experiment. Each rabbit carcass was placed in a wooden box (10X10 X30) cm^3 has openings in four sides for allowing the insects to access into the carcass as well as it allows the researcher to follow the decomposition stages of the carcass. The soil temperature was recorded continuously. The samples were taken with two days interval to the end of the experiment. This sampling design allowed the collection of information on different species of insect succession and their composition on the buried rabbit carcasses at different stages of decomposition, at two depths, in addition duration of each stage during winter and summer seasons.

Sampling and Laboratory work

The 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) the ambient temperatures 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 (3). The second group was kept alive and reared in the laboratory to obtain the adults for sure species identification, by transferring them into a dry glass jar with small amounts of minced chicken liver or beef lung

on a layer of sawdust to allow larvae to be pupate (13).Daily, they were observed and moistened with water. The specimens were observed under a dissecting microscope and identified to order, family and species level according to specific keys (14,15). To confirmation, some samples were sent to the Natural History Museum/University of Baghdad, Iraq.

Statistical Analysis

Study results were analysis as factorial experiments with completely randomized design ,and tested significantly by using L.S.D. test (Least Significant Difference) at level (0.05) for showing results significantly (16).

RESULTS AND DISCUSSION

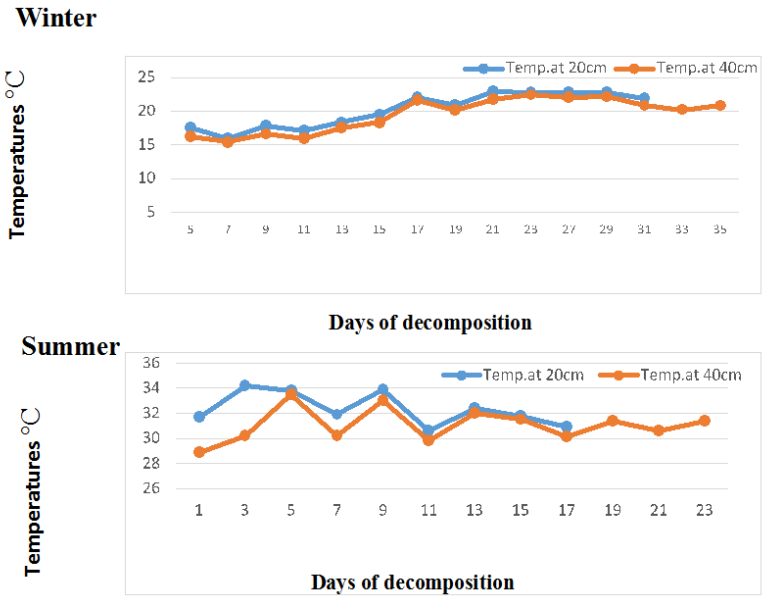
Understanding the stages of decomposition, the colonization of insects and factors that may affect decomposition and colonization are key in determining forensically important information about the carcass. Temperatures range during this experiment were (30.1-31.7) °C in Summer and(17.5-21.9)°C in Winter (Fig.1). During the present study, four stages of decomposition were observed (Table 1 and 2) and (Fig2).

Fresh stage: It begins with the moment of death and continues until the beginning of bloated stage. It was 5, 7 days at Winter and 3,5 days at Summer at two depths 20 and 40 cm respectively.

Bloated stage: The first sign of this stage is the inflation, swelling, and putrefaction of rabbit carcass. This stage is the beginning of the decomposition process. It was 8,10 days at Winter and 6 days at Summer at the two depths 20 and 40 cm respectively. The odor of decay is noticed during this stage. Fluid starts seeping from the carcass.

Decay stage: This stage continued 8,10 days at Winter and 4,6 days at Summer at the two depths 20 and 40 cm respectively. During this stage, the rabbit carcass was deflated and most of the parts were relatively dry .

Dry stage: this stage is the last stage of decomposition. It took 10 ,8 day at Winter and 4,6 days at Summer .It contains only the dry skin and bones of the carcass. The end of this stage is difficult to define due to its long duration. From the above, it is evident that duration of the whole decomposition process varied with the seasons and burying depth underground, for example, at Winter the duration of decomposition at depth 20 cm was 31 days, while at Summer was 17 days. Also, at Winter the duration of decomposition at depth 40 cm was 35 days, while at Summer was 23 days.



Figure(1): Temperatures variations during the experiment at depths 20 and 40 cm during 2015

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The process of decomposition is a continuous and complex and its stages are widely used as a guide to determine the PMI. In this study four stages of decomposition were observed on the rabbit carcasses in buried. The total duration of the decomposition process in depth 40 cm was longer than in depth20 cm. (17) and (18) stated according to the classification which is used , the number of stages was different and the same these stages can be different, according to the area where the carcass is found. (19) state that the duration of decomposition of the carcass that stays above ground was one week, and of a carcass immersed in water were two weeks or buried in the ground were 6-8 weeks. . However, it was pointed out that the number of decomposition stages adopted by a group of worker was different (20). For this reason, several terms on the decomposition stages are existing in the literatures. These classifications were not used in our study because of the different conditions of the experiment.

Table (1): Duration of decomposition stages (day) in buried rabbit carcasses at two depths (cm) during Winter .

Stage / Depth	Fresh	Bloat	Decay	Dry	Total
20 cm	**5**	**8**	**8**	**10**	**31**
40 cm	**7**	**10**	**10**	**8**	**35**

L.S..D. value of level (0.05) for interaction between two depths and different stages of decomposition = 1.061

Table (2): Duration of decomposition stages (day) in buried rabbit carcasses at two depths (cm) during Summer**.**

Stage / Depth	Fresh	Bloat	Decay	Dry	Total
20 cm	**3**	**6**	**4**	**4**	**17**
40 cm	**5**	**6**	**6**	**6**	**23**

L.S.D value of level (0.05) for interaction between two depths and different stage of decomposition = 0.702

Some of the most important factors that can influence the process of decomposition of carcasses in buried are the temperature, insect invaded the carcass, and the burial depth (21).Temperature and humidity have a strong effect on the duration of the decomposition process. Rapid decomposition of the carcass results Higher levels of humidity and temperature while, the opposite place results in slower carcass decomposition (22). The variations in temperature and relative humidity are mainly associated with the seasons of the year. However, several studies have demonstrated a seasonal variation on the carcass. (23, 24).

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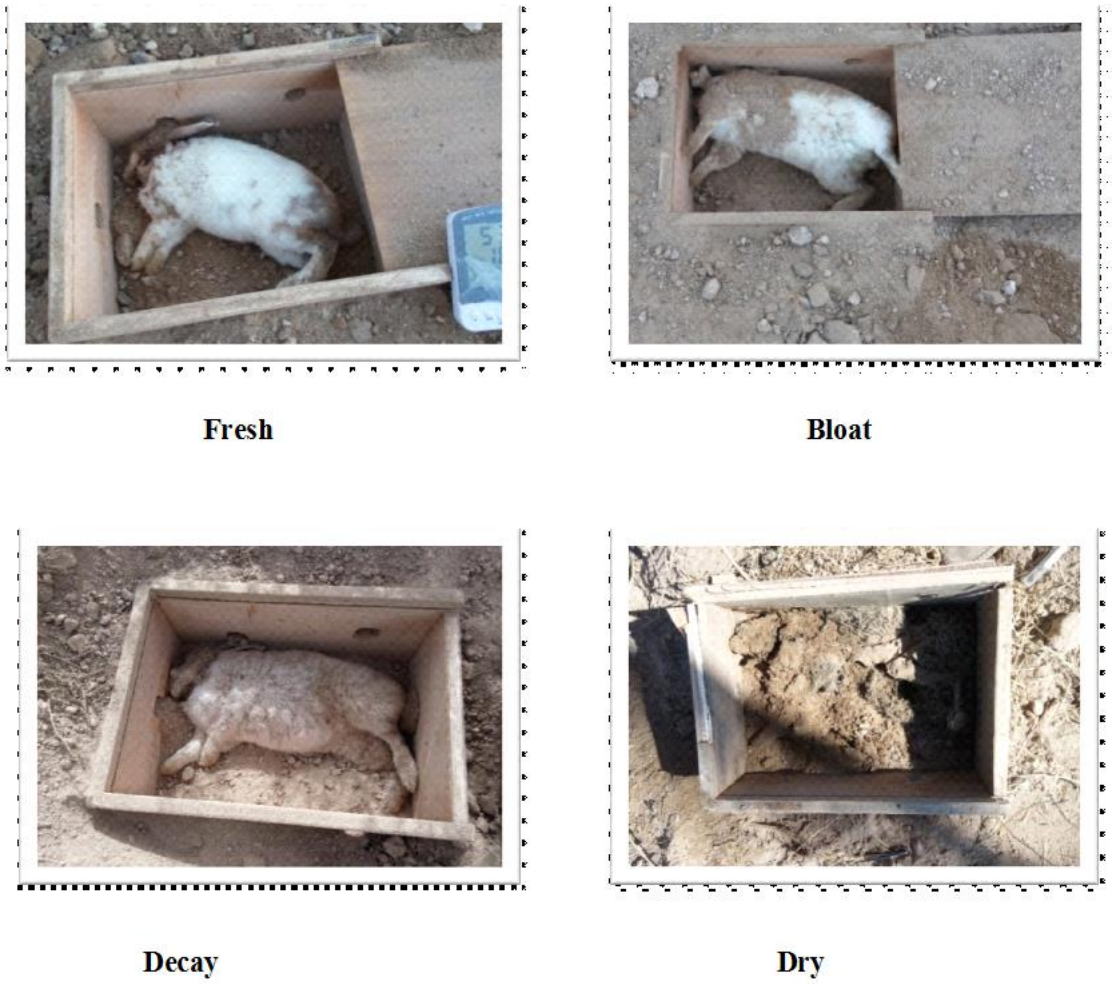

Fresh stage 29/1 to 1/2/20015 **Bloat stage 2/2/20015**

Figure (2): Stages of decomposition process on buried rabbit carcass at depth 20 cm

Also, the type of soil reduces the activity of aerobic bacteria, making the process of decomposition slower, With the moment of death the fresh stage is started, the autolysis process occurs in the rabbit carcass which is destroying the complex proteins and carbohydrates and converted it to the simplest chemical compounds. While, in the bloated stage the intestine produced gases due to the action of anaerobic bacteria , resulting the inflation of carcass (25).

Succession pattern of insects at depth 20 and 40 cm during Winter

During this experiment, two dipteran species were observed *Ch. albiceps* belong to *Calliphoridae* family, and *S. africa* belong to Sarcophagidae family in addition, two species belong to the order Coleoptera *D.maculates* and *Saprinus sp*. as well as one species belong to Hymenoptera *Cataglyphis sp*.(Fig. 3 , 4 , 5 and 6). Succession pattern of insect orders observed on buried rabbit carcasses at depth 20 and 40 cm during Winter which are presented in Fig.(3)and (4). **In the fresh stage:** larvae of Diptera and adults of coleopteran species, *Cataglyphis sp.* were not observed at depth 20 and 40 cm. **In the bloated stage:** Larvae of *Ch. albiceps* at depth 20 cm. were collected on the rabbit carcass from the 7^{th} -13^{th}day of the decomposition and *S. africa* were collected from the 9^{th}-13^{th} day, the most abundance were to the first species. The *Cataglyphis* sp .started in appearance during this stage at depth 20 cm (Fig.3). At depth 40, Larvae of *Ch. albiceps*, and *S. africa* were observed in the end of this stage from 10^{th} - 17^{th} day. In addition the appearance of *Cataglyphis* sp.(Fig 4).

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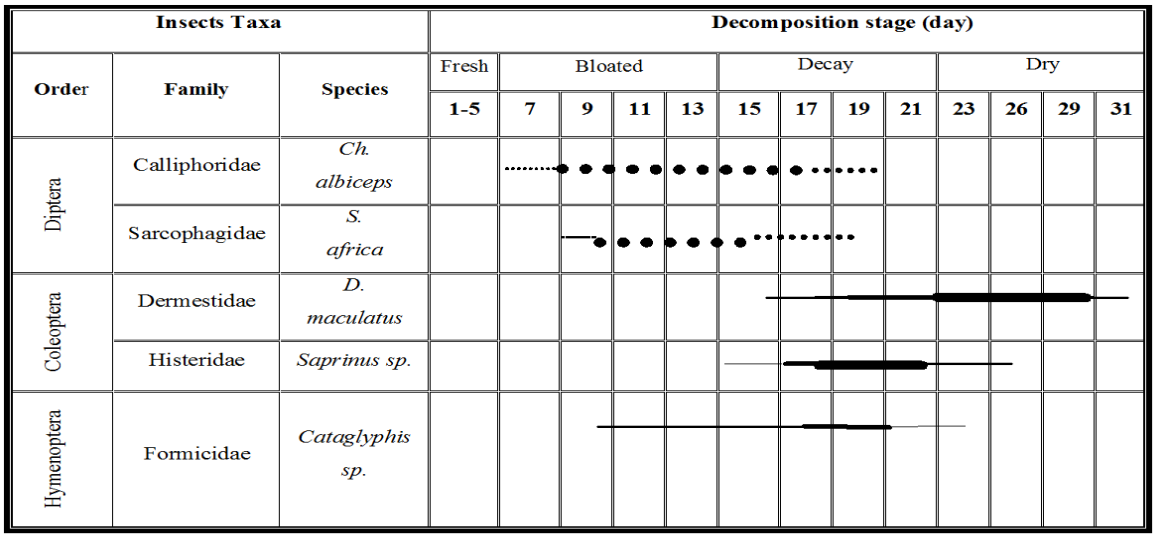

Figure: (3) Succession pattern of insects collected from buried rabbit carcass at depth 20cm. during Winter, 2015

It represents a very small number of It represents a very small number of beetles and ant
It represents a small number of insects beetles and ant
It represents a moderate numbers of beetles and ant
It represents a large number of beetles and ant
It represents the larva of Diptera

In the decay stage this stage showed the presence of all species of insects but in different ratio. In addition to the appearance of Larvae of *Ch. albiceps* and *S. africa*, Second wave of insect begin to visit the rabbit carcasses, adults of *D. maculates* and *Saprinus sp.* observed at two depth .The abundance of all insect species were more abundance at depth 20cm than 40 cm (Fig. 3) and *Saprinus sp.* was most abundant at two depths. Also, The *Cataglyphis* sp. continued in appeared during this stage at two depths. **In dry stage**, when the odor disappeared and the nutrient matter became very little, the larva of flies began to decline and changed to the pupae at two depths. Also, *D. maculatus* and *Saprinus sp.* were continued to appear during this stage at two depths but *D. maculates* was most abundance especially in 23^{th} -29^{th} day at depth 20 cm (Fig. 3) and in 27^{th}-29^{th} day at depth 40 cm (Fig. 4). As well the *Cataglyphis sp*. it was viewed during the 1^{st} day of this stage at depth 20 cm but it disappeared with the beginning of this stage at depth 40 cm.

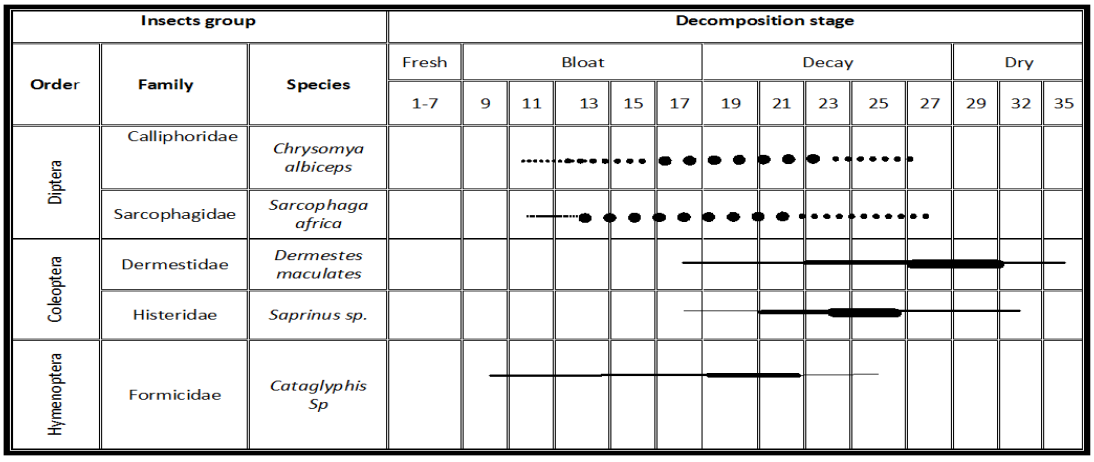

Figure (4): Succession pattern of insects collected from buried rabbit carcass at depth 40cm. during Winter, 2015

It represents a very small number of It represents a very small number of beetles and ant
It represents a small number of insects beetles and ant
It represents a moderate numbers of beetles and ant
It represents a large number of beetles and ant
It represents the larva of Diptera

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Succession pattern of insects during Summer

Succession pattern of insect orders observed on buried rabbit carcasses at depth 20 and 40 cm during summer has been given in Fig. (5) and (6).**In the fresh stage:** Dipteran flies, coleopteran species and the ants *Cataglyphis* sp. were not observed at depth 20 and 40 cm .**In the bloated stage:** Larvae of *Ch. albiceps* and *S. africa* at depth 20 cm were collected from the 5th-9thday of the decomposition. *Ch. albiceps* was the most abundance compared with *S. africa* at depth 20 cm Fig. (5). On the contrary, at depth 40 cm, the larvae of *S. africa* was more abundant observed but *S. africa* was more abundant than *Ch. albiceps* Fig. (6) Coleopteran species were observed at the end of this stage at depth 20 and 40 cm. The *Cataglyphis sp.* began to appear with the beginning this stage at depth 20 and 40 cm.

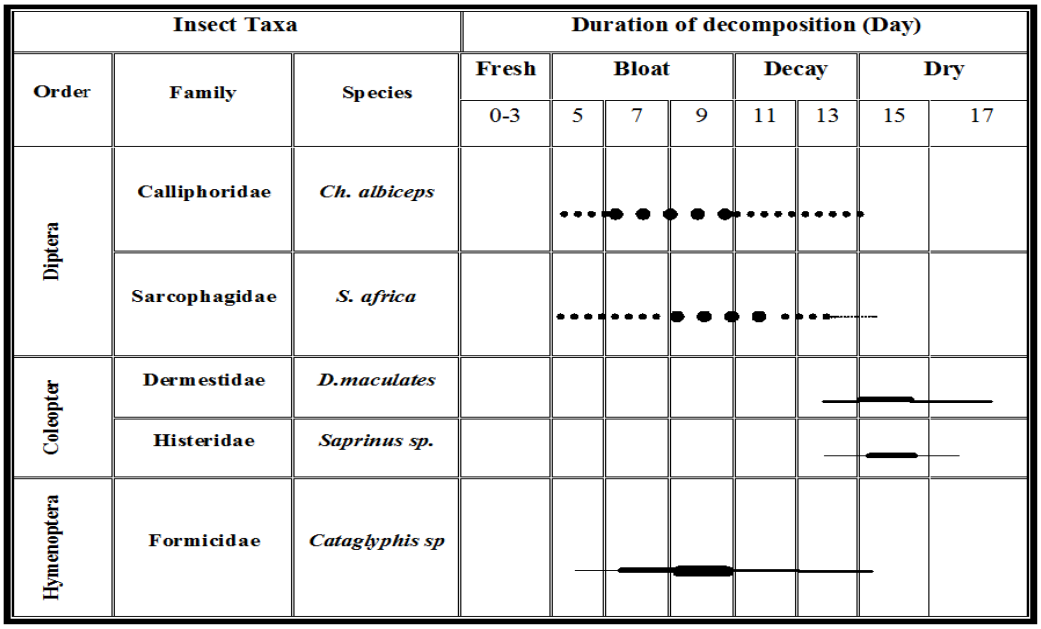

Figure (5): Succession pattern of insects collected from buried rabbit carcass at depth 20cm. during Summer, 2015

It represents a very small number of beetles and ant
It represents the presence of a small number of beetles and ant
It represents the presence of moderate numbers of beetles and ant
It represents the presence of a large number of beetles and ant
It represents to the larva of fly

In the decay stage, it was showed the presence of species of insects but in different ratio. The abundance of *Ch. albiceps* and *S. africa* larvae began to decrease at depth 20 and 40 cm. and second wave of insects(Coleoptera)continued to invade the rabbit carcass. The abundance of the two species of Coleopteran approximately are equal at depth 20 cm (Fig. 5),but at depth 40 cm the abundance of *D. maculates* was more than *Saprinus sp*. (Fig. 6). Also, the adult of *Cataglyphis* sp. continued to appear during this stage at two depths. **In dry stage,** when the odor disappeared and the nutrient matter became very little, the larva of flies began to decline and changed to the pupae at two depths .As well as, *D. maculatus* and *Saprinus sp.* were continued to appear during this stage at two depths but *D. maculatus* was most abundant especially in 15th-17th day at depth 20 cm(Fig. 5)and in 19th-23th day at depth 40 cm(Fig. 6). As for the *Cataglyphis* sp. it was viewed during this experiment during 5th-15th day at depth 20 cm and from 7th-19thday at depth 40 cm.

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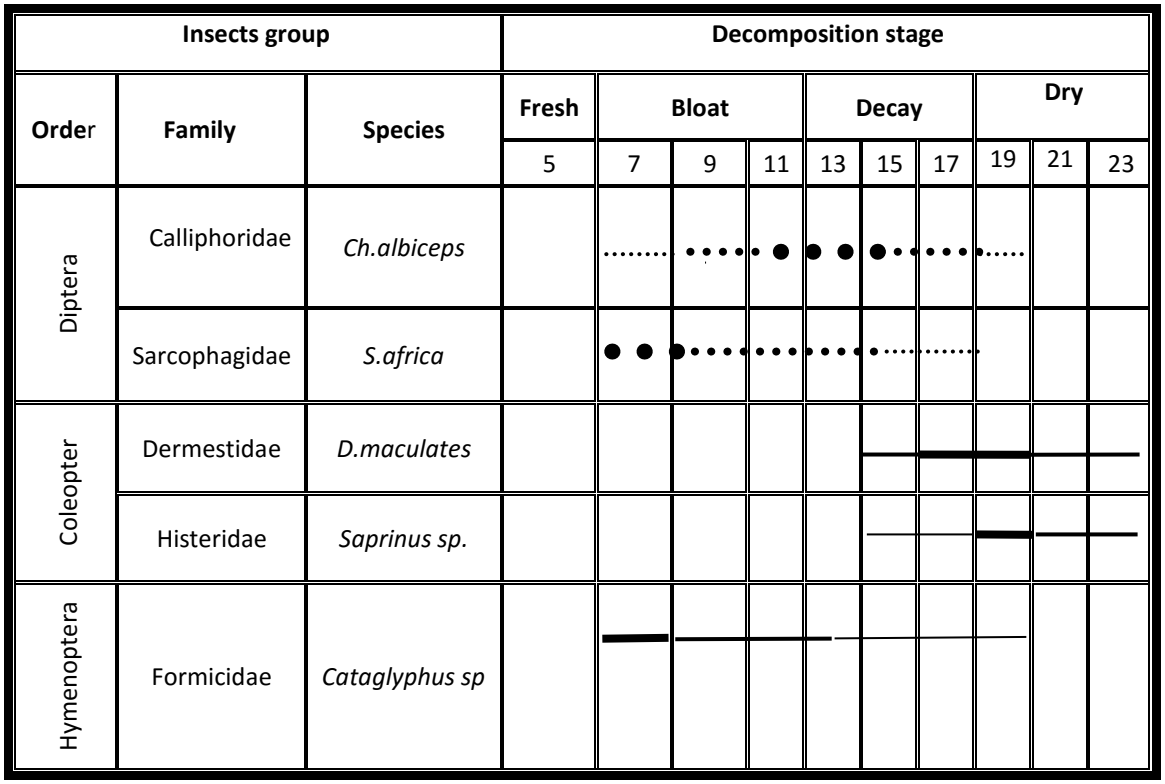

Figure (6): Succession pattern of insects collected from buried rabbit carcass at depth 40cm. during Summer, 2015

It represents a very small number of insects
It represents the presence of a small number of insects
It represents the presence of moderate numbers of insects
It represents the presence of a large number of insects
It represents to the larva of Diptera

In this study two dipteran species were collected *Ch. albiceps* and *S. africa* in addition, two species belongs to the order Coleoptera *D. maculatus* and *Saprinus sp*. as well as one species belong to Hymenoptera *Cataglyphis sp*. In Reports describing the insect succession pattern on buried carcasses (including human corpses) at several habitats, Diptera was dominated, especially species of Muscidae, Phoridae and Sarcophagidae utilizing the carcass as a food source (23,26). Many of the insects associated with above ground carcasses are restricted from colonizing a buried carcass (9). However, it was observed the blow flies *Calliphora vomitoria*, *C. vicina* and *L. sericata* possess a limited capacity to invade baits of buried pig liver in loose soil at depth 10 and 20 cm (27). These families were present on carcasses in buried with different seasonal faunal composition and played an important role in the decomposition of rabbit carcass as major factors in carcass decomposition and significance to forensic entomology.

In this study, the carcasses were buried in depth 20 and 40 cm. These depths were chosen based on the methodology used by (28), who states these depths are a common depths for burying carcasses. It was founded that the fauna of insects on a buried carcass may be differed from that found on an exposed carcass as well; the collection specimens of insects from a buried body should proceed in a similar way to those descriptions to the exposed carcass (29). In this study, the larvae of flies observed at two depths 20 and 40 cm in Winter and Summer. (30) point out that species of *Muscina*(Muscidae) and *Eumacronychia persolla* will invade carcass in shallow graves and species of scuttle flies (Phoridae) will invade bodies buried under 1–2m of soil .While, (27) stated even a shallow burial at depth 10–20 cm will deter most blowfly species. In study of (24) *Ch. albiceps* invested two cases which were buried under the ground, the mechanism by which Phorid flies could reach buried corpses could be explained as follows: adult phorid flies of genera *Conicera* and *Metopina* reach the soil depth of 50 cm in four days. In addition, adults of the parasitic wasps of the families Braconidae and proctotrupidae occur in soil depth of at least 50cm. Conversely, bigger fly species, the larvae of which dominate in carcasses covered with a thin layer of soil, e.g., *Muscina* species lay their eggs on the soil surface,

and the young larvae migrate into the carrion through the soil (31). It was found that Sarcophagidae invaded the buried rabbit carcasses at depth10-20 cm in March and April (32). (33) founded the larvae of Sarcophagidae were demonstrated under 25°C temperature but (24) found a significant growth of Sarcophagidae was observed at 25°C. (34) founded that the insect succession on buried pig carcass at 30 cm proceeded with flesh and muscid flies being the first to colonize, followed by blow flies. In addition, these insects were able to colonize carcasses at 60 cm and *Hydrotaea sp*. and *Megaselia scalaris* (Diptera: Phoridae) were collected after7 days of burial. (35) collected a total 10 beetle species belonging to 6 families i.e. *Saprinus pensylvanicus*, *Saprinus sp*., *D. maculatus* when studying the successional pattern of insects on pork piece used as research model which was clothed with the cotton cloth and buried at a depth of 30 cm in the forest area of Punjab-India. Many of studies on buried human corpses and pig carcasses have identify various species of Coleoptera, including Staphylinidae, Histeridae, Carabidae, Leiodidae, Dermestidae and Silphidae (28,36) Many parameters like the manner of storage of a corpse, habitat, or time of year (season), will also affect the composition community of the insects on the carcass. When death occurred, these parameters may help to determine the season and month of the year when crime occurred (32,37).

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