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# Influence of Seasons on the Process of Colonization by Insects of Pig Corpses Exposed to the Open Air in Sub-Sudanese Zone of Côte d'Ivoire

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**Abstract:** ***Necrophagous insects are very useful bioindicators in determining of post-mortem interval. The objective of this work was to determine the influence of seasons in the process of colonization by insects of a corpse exposed in the open air. Three pigs of 50kg each, were used as bait on each season in the sub-sudanese zone of Côte-d'Ivoire. Harvesting of adult insects was done by a filleting net. Samples of eggs, larvae and pupae were taken from the cadavers and then breeded in the laboratory. The insects collected were Coleoptera and Diptera. Three groups of insects were identified in the dry season and five in the rainy season. The the families of Calliphoridae, Muscidae, Sarcophagidae, Cleridae, Histeridae and Dermestidae were the first group in the two seasons appeared at fresh corpse, swelling and active decomposition. In both seasons, the 2nd group was composed of Cleridae, Histeridae and Dermestidae with the exception of Muscidae and Fanniidae, which were collected only during the rainy season. The 3th group was constituted of Tenebrionidae and Dermestidae in the dry season and Calliphoridae, Stratiomyidae, Piophilidae, Muscidae, Histeridae, Cleridae and Dermestidae in rainy season. These insects were captured during the active decomposition and advanced decomposition stages. The Piophilidae, Stratiomyidae,The appearance of Scarabaeidae and Tenebrionidae form the observed in advanced decomposition stages and skeletonization were the 4th group in the rainy season. The 5th group was composed of Piophilidae, Stratiomyidae, Dermestidae and Tenebrionidae. These insect families could serve as indicators in forensic entomology expertise.***

**Keywords:** Corpses, Insects, Colonization, Côte d'Ivoire

## 1. Introduction

Insects are very useful bio-indicators for estimating the post-mortem interval (PMI) at the time the discovery of a corpse (Gennard, 2007; Dekeirsschieter, 2012). To solve a murder, investigators need to know the causes and the date of death. If the short post-mortem estimate is based on the use of the first Diptera colonizer of the corpses, that of the post-mortem interval medium and long may rely on how insects succeed on a corpse in its complete decomposition. In many forensic studies, it was demonstrated that the succession of different groups of insects on a corpse is closely linked to the five or six stages of decomposition, which vary by region, climate and other factors intrinsic to the corpse (Mégnin, 1894; Byrd & Castner, 2001 ; Silahuddin *et al*., 2015 ; Dao *et al.*, 2018) . In addition, work on the process of colonization by necrophagous insects ofpig corpse exposed to the open air guinean zone of Côte d'Ivoire were made by (Koffi *et al.*, 2017). The literature does not mention any similar study in the sub-sudanese zone of Côte d'Ivoire. The objective of this work was to identify the different groups of insects involved in the process of colonization of pig corpse exposed in the open air in the sub-sudanese zone during the rainy and dry seasons.

## 2. Material and Methods

### 2.1 Study site

The work took place in the town of Korhogo located in the sub-sudanesezone, northern part of Côte d'Ivoire. The botanical garden of Peleforo Gon Coulibaly University (9°26'N - 5°38' W, altitude 380m) was chosen for the experiments.

### 2.2 Experimental climatic conditions

A thermo-hygrometer of brand and model HMI-172SI and a rain gauge were used for the respective taking of the temperature and the relative humidity and the pluviometry at the study site. The sub-sudanese zone is characterized by two seasons : the dry season and the rainy season. The temperature oscillated between 25 and 36°C with an average of 30.8 ± 3.7°C during the dry season. In the rainy season, the temperature varied from 24.6 to 33.2°C with an average of 26.7°C ± 4.2°C. As for the relative humidity, it varied from 14 to 63% with an average of 46.53 ± 15.57% for the dry season and from 68 to 86% with an average of 76.85% in rainy season. In the dry season, rainfall was very low or even nil. The values oscillated between 0 and 75 mm while in the rainy season, it varied from 46 to 310 mm.

### 2.3 Experimental device and exposure of corpses

The pig corpses were exposed in wire cages (L = 1.5 m, l = 0.80 m and h = 0.70 m) (Dao *et al.*, 2018) and allowed all the insects to have access to the carcasses. However, the wire has prevented vertebrate scavengers to access the corpses. Three pig carcasses representing three replicates were used for each exposure series. A first exposure was made during the dry season from November 7, 2017 to February 28, 2018

including the harmattan period. A second was carried out during the rainy season from 05 June to 15 August 2018.

### 2.4 Followingand harvesting necrophagous insects

The following of corpses exposed to the open air was done daily for the duration of the complete decomposition. The direct observations were made at the different parts of the corpse (faces hidden, inner ears, anal orifice, mouth) every 30 minutes. Thus, necrophagous insects were harvested according to three methods :

- Method 1 : Egg samples were collected at each stage of decomposition. The eggs collected were incubated in the laboratory and the larvae obtained were breeded on pig substrate (liver or striated muscle) until emergence of adults. The emerged flies were killed with ether or gaseous carbon dioxide ($CO_2$), sorted, identified and counted. The purpose of egg collection and larval rearing was to confirm that the adult species harvested at the different traps were effectively necrophagous insects on the one hand and to determine the precise period of colonization of the carcasses on the other hand.

- Method 2 : Samples of insect larvae found at each stage of decomposition were collected. Diptera pupae were also harvested from and/or near cadavers using soft forceps. The collected larvae were breeded and pupae collected were directly introduced into the emergence boxes to obtain adult flies in the laboratory.

- Method 3 : Flying insects were captured with a fanning net. The catches were made three times a day, at 9 o'clock in the morning, at 12 o'clock and in the evening at 6 o'clock.

The insect identification was made using a binocular magnifying glass brand and model "Optika LAB20" version 4.0 and various identification keys (Wyss & Cherix, 2006 ; Whitworth, 2010 **;** Szpila & Villet, 2011 ; Szpila, 2014 ; Irish *et al*., 2014 ; Rochefort *et al.*,2015 ; Vairo *et al*., 2015; Alikhan *et al*., 2016).

### 2.5 Statistical analyzes

Data processing was carried out by STATISTICA Version 7.1. The Student's t-test allowed the comparison of samples from both seasons and the test from Newman Keuls at the 5% threshold allowed to separate the averages the numbers of the different families of necrophagous insects collected.

## 3. Results and discussion

Two orders of necrophagous insects (Diptera and Coleoptera) were collected. The Diptera were divided into six families and the Coleoptera into five families.

### 3.1 Abundance of individualsin the different families of Diptera

Individuals from the family of Calliphoridae were more abundant in the rainy season with an average of 8181.66 ± 283.6 individuals (Table 1).

The t-test of Studentat the 5% threshold (t = 16.2051, df = 4 ; P <0.0001) indicated significant differences between the average number obtained for this family during the both seasons. Regarding the family of Sarcophagidae, the separation test of Newman-Keuls at 5% threshold has revealed that more individuals were collected in the rainy season with an average number of 1348.66 ± 154.21. The t-test of Student at the 5% threshold indicated significant differences between the average number obtained for this family during the dry and rainy seasons (t = 4.5160; df = 4 ; P < 0.001) (Table 1).

The Newman-Keuls separation test at the 5% threshold revealed that individuals of Muscidae were less abundant in the dry season ( t = 5.0215 ; df = 4 ; P < 0.05) (Table 1).
The families of Piophilidae and Stratiomyidae have been collected in rainy season respectively withaverages number of 2727.66 ± 231 and 2989.33 ± 204 individuals. The t-test of Student at the 5% threshold revealed significant differences between mean Piophilidae numbers (t = 13.7680, df = 4 ; P <0.0001) and those of Stratiomyidae (t = 12.9716 ; df = 4 ; P = 0.0001) recorded during both seasons (Table 1).

**Table 1 :** Abundance of individuals of Diptera families harvested in dry and rainy seasons

| Study period | Calliphoridae | Muscidae | Fanniidae | Sarcophagidae | Stratiomyidae | Piophilidae |
|---|---|---|---|---|---|---|
| Dry season | 2999 ± 14.22$^b$ | 467.66 ± 40$^b$ | 0.00 ± 0.00$^b$ | 197 ± 14.2$^b$ | 0.00 ± 0.00$^b$ | 0.00 ± 0.00$^b$ |
| Rainy season | 8181.66 ± 283.6$^a$ | 1020.66 ± 102$^a$ | 168.66 ± 57.2$^a$ | 1348.66 ± 1 54.6$^a$ | 2989.33 ± 231$^a$ | 2727.66 ± 204$^a$ |
| t | 16.2051 | 5.0215 | 2.9458 | 4.51600 | 12.9716 | 13.7680 |
| df | 4 | 4 | 4 | 4 | 4 | 4 |
| p | < 0.0001 | < 0.05 | < 0.05 | < 0.001 | < 0.0001 | < 0.0001 |

*Note : The figures followed by the same letter in one column are not significantly different according to the Newman Keuls test at the 5% threshold.*

### 3.2 Abundance of individualsin differentfamiliesof Coleoptera

The Coleoptera species collected during the dry and rainy seasons were grouped into four families. The appearance of these species of beetle is made of three different ways :

- 1st case : populations of Histeridae,of Dermestidae and of Tenebrionidae have during the transition from the dry season (November 2017- February 2018) in the rainy season (June - August 2018). The t-test of Student at the 5% threshold indicated significant differences between the average number of individuals of Histeridae (t = -10.3229; df = 4 ; P < 0.001), Dermestidae (t = - 7.8890 ; df = 4 ; P < 0.05) and

Tenebrionidae (t = - 4.6301 ; df = 4 ; P < 0.05) recorded during both seasons (Table 2).

- 2nd case : a few individuals of the family of Scarabaeidae were collected in the rainy season but in the dry season, none was harvested. The t-test of Student at the threshold of 5% does not indicated difference between the average number obtained for this family during the two seasons (t = 2.0000; df = 4 ; P > 0.05) (Table 2).

- 3rd case : the individuals belonging to the family of Cleridae have been abundant both in the dry season than in the rainy season. Thet-test of Student at the 5% threshold doesn't indicated difference between the numbers of this family listed during the dry and rainy seasons (t = - 0.9629 ; df = 4 ; P > 0.0 5) (Table 2).

**Table 2:** Abundance of individuals of Coleoptera families harvested in the dry and rainy seasons

| Study period | Histeridae | Cleridae | Dermestidae | Scarabaeidae | Tenebrionidae |
|---|---|---|---|---|---|
| Dry season | 942 ± 57.7[b] | 163 ± 35.25[a] | 562 ± 54.5[a] | 0.00 ± 0.00[a] | 16.33 ± 1.7[a] |
| Rainy season | 289.33 ± 25.7[a] | 114.33 ± 36.2[a] | 24.66 ± 62.23[b] | 0.66 ± 0.003[a] | 0.00 ± 0.00[a] |
| t | -10.3229 | -0.9629 | -7.8890 | 2.0000 | - 4.6301 |
| df | 4 | 4 | 4 | 4 | 4 |
| p | < 0.00 1 | > 0.05 | < 0.0 5 | > 0.05 | < 0.0 5 |

*Note : The figures followed by the same letter in one column are not significantly different according to the Newman Keuls test at the 5% threshold.*

**3.3.Sequence of appearance of different groups of necrophagous insects on the pig's corpse exposed to the open air**

**3.3.1.In the dry season**

The colonization by insects of pig carcasses exposed to the open air was done in a sequence constituted of three groups :

- the 1st group wasconstituted of Diptera of the families of Calliphoridae, of Muscidae and of Sarcophagidae in one hand and Coleoptera belonging to the families of Cleridae, Histeridae and Dermestidae in other hand. The species of the insect families were recorded in the stagesfresh corpse (FC), swelling (Sw) and active decomposition (AcD) between the 1st and 6th postmortem day (Figure 1). The first colonizations were done by the family of Calliphoridae 24 hours after the killing of the pigs and the exhibition of the cadavers. In this first group, individuals of the family of Calliphoridae were the most numerous (Figure 1).

- the 2nd group was composed by species of Cleridae, Histeridae and Dermestidae. The species of these families were maintained after the disappearance of Calliphoridae, Muscidae and Sarcophagidae in active decomposition phase (AcD). The families of these Coleoptera increased in number at the stage of active decomposition except the numbers of Histeridae which decreased (Figure 1). The species of these three families of Coleoptera remained on the carcasses during the advanced decomposition phase between 9th and 60th post-mortem day for the Histeridae's family and the 50th day for the Cleridae's family (Figure 1).

- after the disappearance of Histeridae and Cleridae, the group 3 has formed with the arrival of Tenebrionidae the at the 90th day which were added to Dermestidae. These two families remained at the end of the advanced decomposition phase and in the beginning of skeletalization phase (Figure 1).

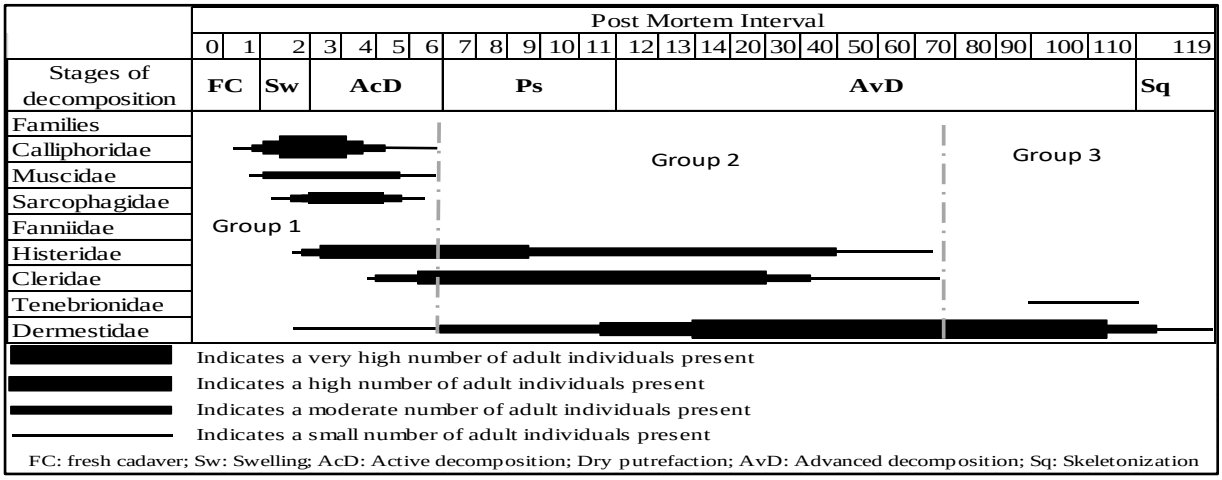


**Figure 1 :** Period of occupation of the corpse exposed in the dry season by necrophagousinsects

**3.3.2 Inrainy season**

The colonization of pig carcasses exposed to the open air by insects was done in a sequence constituted of four groups:

- Between 1st and 9th day post-mortem, the pigs' carcasses were colonized by a first group constitutedbythe families of Calliphoridae, of Muscidae, of Sarcophagidae and of Fanniidae in one hand and Coleoptera Cleridae, Histeridae and Dermestidae of somewhere else. Individuals of families

of these two orders have appeared in stage of fresh cadaver (FC), swelling (Sw) and active decomposition (AcD) (Figure 2). The family of Calliphoridae disappeared on 9th postmortem day (Figure 2).

- after the disappearance of the family Calliphoridae at the stage of active decomposition, the species of Muscidae, of Sarcophagidae,Fanniidae, Cleridae, Histeridae and Dermestidae have maintained to constitute the 2nd group (Figure 2).

- in the 12th day postmortem, the familiesof Stratiomyidae and Piophilidae have appeared. These insects were added to the families of the 2nd group to form the 3thgroup at the end of active decomposition(AcD) and advanced decomposition stage (DAv). The activity of this group was included between the 12th and the 20thpost-mortem day. When the numbers of families of Muscidae, of Histeridae and of Cleridae were decreasingwhen those of Dermestidae, of Stratiomyidae and Piophilidae increased (Figure 2).

- the Scarabaeidae family was appeared at the 40thpost-mortem day and was added to the families of Dermestidae, of Stratiomyidae and of Piophilidae to form the 4th group of the stage ofadvanced decomposition and skeletonization stage (Figure 2).

- in advanced decomposition, Tenebrionidae appeared at the 90th day post mortem were added to the families of Dermestidae, of Stratiomyidae and of Piophilidae to form the 5th group at the end of advanced decomposition (Figure 2). The Piophilidae and Stratiomyidae have respectively appeared on 12th and 14th post-mortem day and disappeared on the 100thandon the 111thpostmortem day respectively (Figure 2).

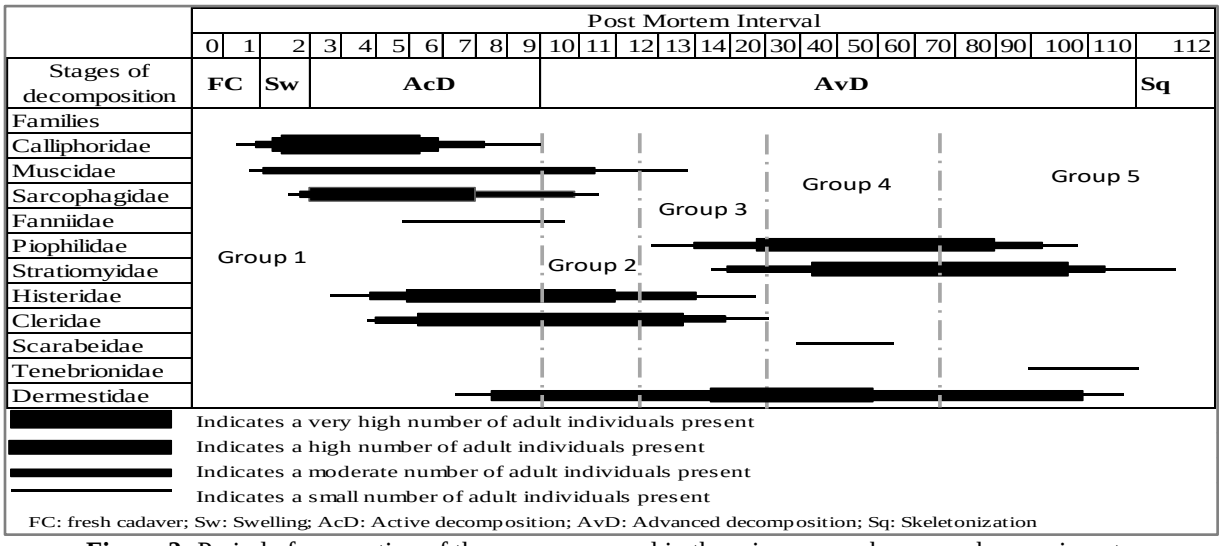


**Figure 2:** Period of occupation of the corpse exposed in the rainy season by necrophagous insects

The study of the colonization process by necrophagous insects of pig corpses exposed in the open air in the sub-sudanese zone of Côte d'Ivoire, allowed to collect mainly Diptera and Coleoptera.

In the dry season, three groups of necrophagous insects were observed on the exposed corpses. The 1st groupwas constitutedby Diptera of the families of Calliphoridae,of Muscidae and Sarcophagidae one hand and Coleoptera belonging to the families of Cleridae,of Histeridae and of Dermestidae other hand. Species of these insect families have been observed in fresh cadaver, swelling and active decomposition stages. These observations are different from those of Koffi *etal*., ( 2017a) who found that the first group of insects to colonize open air cadavers were only the Diptera belonging to the families of Calliphoridae, of Muscidae and of Sarcophagidae. Indeed, the appearance of this first group of insects at these stages of decomposition could be explained by the fact that these insects would have powerful olfactory devices allowing them to detect very weak smells at several hundred meters. This argument is similar to that of (Picimbon, 2002). The presence of Coleopteran families on corpses exposed to the open air during the dry season in the sub-sudanese zone is due to the fact that the high temperatures have liquefied the fats of corpses causing odors of rancidity. These arguments are similar to those of (Mayer & Vasconcelos, 2013).

The presence of the beetle families could also be due to the presence of Dpiteran eggs and larvae of 1st and 2nd stage on the corpses. Indeed, some species of Coleoptera such as Dermestidae, Histeridae etc. are fond of eggs and larvae of Diptera. This argument is similar to that of Campobasso *et al.*, (2001) who reported that individuals of Histeridae were fond of Diptera eggs and larvae, in their work on factors affecting the decay and colonization of corpses by the necrophagous Diptera. The 2ndgroup was composed of species of Cleridae, of Histeridae and of Dermestidae. These families have maintainedafter the disappearance of the first Dipteran in the active decomposition stage. The species of these three families remained on the carcasses during the advanced decompositionstage between 9th and 60th post-mortem day for Histeridae and the 50th post-mortem day for Cleridae. The disappearance of Cleridae and Histeridae repectivement the 50th and 60th day post-mortem, was due to drying of the corpses by the very low relative humidity and lack of eggs and larvae of Diptera (Dao *et al.*, 2018). This finding is also similar with that of (Campobasso *et al.*, 2001). The Dermestidae remained on the corpses until the advanced phase of decomposition and at the beginning of

skeletonization. The persistence of the Dermestidae family on the carcasses was due to the drying of the carcasses by the high intensities of insolation and the low relative humidity. Effect, adults and larvae of species of this family feed on dry animal debris (Dao *et al.*, 2018).

In the rainy season, observations made on pig carcasses exposed to the open air allowed to distinguish five groups of necrophagous insects. This finding is similar to that of Koffi *et al*., (2017a) who, during their work madein the guinean zone of Côte d'Ivoire, distinguished four groups of insects colonizing corpses exposed to the open air. The 1st group was identical to that of the dry season. The presence of these insects in these stages of decomposition would therefore be due to their olfactory system. This observation is due to the strong odors released by the corpses at these stages of decomposition. Studies have shown that egg-laying by Dipteran females is caused mainly by the distinctive odors of the decaying corpse (Hall *etal*.,1995; Rodriguez & Bass, 1985) and that the intensity of attraction depends largely on the concentration of odors (Barton-Browne, 1960). The disappearance at the 9thpost mortem daay of Calliphoridae s' family let appear the 2nd group constituted of species families of Muscidae, of Fanniidae, of Cleridae, of Histeridae and of Dermestidae. This finding is similar to that of (Koffi *et al*., 2017a). At this stage of decomposition, corpses swarmed with Diptera larvae at various stages of development. These Diptera larvae were prey for some Coleoptera (Campobasso *et al.*,2001 ; Dao *etal.*, 2018). In the 12th day after death, the families of Stratiomyidae and Piophilidae have appeared. These insects, which were rare in the dry season, were very abundant on corpses during the rainy season (Lord *etal.*, 1994 ; Turchetto, 2000 ; Tomberlin & Sheppard, 2005;Dao *et al*., 2019). This lack of activity of Piophilidae and Stratiomyidae in the dry season could be explained by the desiccation of cadavers, due to high heat (temperatures greater than or equal to 36°C) and low relative humidity (18 to 25%) during the harmattan period) (Dao *etal*., 2019). The high abundance of Piophilidae and Stratiomyidae in the rainy season can be due to advanced decomposition of corpses remaining moist, which would favor the proper development of larvae of these families (Turchetto, 2000 ; Tomberlin *et al*., 2009;Koffi *etal*., 2017b). The corpse ecosystem was richer in insect species in the rainy season than in the dry season. The richness observed in the rainy season can be explained by the high relative humidity and precipitations on one hand and the humidity of the corpses on other hand. These factors favor the colonization of the corpse by various insects but also allow these insects to complete their reproductive activity. The succession of necrophagous insects on cadavers could also be explained by the fact that the first colonizers release nutrients through their activities to the following species on the one hand and by their diets on the other hand. According to Warren & Anderson, (2008) some such as Calliphoridae are necrophages, others such as Histeridae, Dermestidae are predators.

## 4. Conclusion

Pig carcasses exposed in the open air in the sub-sudanese zone of Côte d'Ivoire were colonized by necrophagous insects belonging to two Orders, namely Diptera and Coleoptera.

In the dry season, three groups of insects were found: the 1rstgroup consituted of Calliphoridae, Muscidae and Sarcophagidae on the one hand and Ceridae, Histeridae and Dermestidae other hand. Species from these insect families were observed at fresh cadaver stages, swelling and active decomposition ; the second group was composed by solely of Coleoptera of the families of Cleridae, of Histeridae and of Dermestidae, after the disappearance of the Diptera of the first group. These Coleoptera were maintained at the stages of active decomposition, advanced decomposition and dry putrefaction. The third group constituted of Tenebrionidae which were added to the Dermestidae of the second group. These insects were observed during the advanced advanced decomposition stage and skeletonization of the corpses.

In the rainy season, five groups of insects have been identified : Insect families appeared in the 1st group were identical to those of the first group of the dry season, with the exception of the family of Fanniidae. The disappearance of Calliphoridae allowed the formation of the 2ndgroup with Diptera Muscidae and Fanniidae on the one hand and Coleoptera Cleridae, Histeridae and Dermestidae on the other hand. The Stratiomyidae and Piophilidae appeared between 12th and 14th post-mortem days, were added to the families of the 2nd group to form the 3rd group. After the disappearance of Histeridae and Cleridae, the Stratiomyidae, Piophilidae and Dermestidae have remained on the carcasses to form the 4th group with the appearance of the family of Scarabaeidae. The 5th group was constituted of Diptera Stratiomyidae and Piophilidae in the one hand and Coleoptera and Dermestidae Tenebrionidae in the other hand.

## 5. Declaration of Interests

The authors declare that they have no conflicts of interest in relation to this article.

## 6. Acknowledgment

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