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Feugang Youmessi Francis Dupont
Department of Animal Biology and Physiology, University of Yaounde 1. Po. Box: 812, Yaounde, Cameroon

Djonga Gnoua
Department of Animal Biology and Physiology, University of Yaounde 1. Po. Box: 812, Yaounde, Cameroon

Corresponding Author:
Feugang Youmessi Francis Dupont
Department of Animal Biology and Physiology, University of Yaounde 1. Po.Box: 812, Yaounde, Cameroon

Necrophagous entomofauna and evaluation of his parasitoïdism level during the dry season within the campus of the University of Yaounde 1

Feugang Youmessi Francis Dupont and Djonga Gnoua

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Abstract
After death of animal, necrophagous insect invade the death boby for many reasons. These carcasses provide a nourishing substrate, eggs laying area, a breeding site, a refuge for this specific group of arthropod that can be used as valuable auxiliaries of justice. These organisms can help to determine the time elapse since death, the movement of the corpse after crime for Human beings, the abandon of elderly and the cause of death. The exploitation of these insect for criminal purpose is delicate since they are subject of attack by parasitoïds that can affect their life cycle witch is the main key of the use of insect on forensic research. The aim of this research work is to collected necrophagous insects and assess their level of parasitoïdism during their larval rearing in the laboratory under natural ambient air conditions during the dry season. The experiment was performed on two rat carcasses *Rattus norvegicus* at the field of the University of Yaounde 1. These cadavers were placed inside a wooden cage where the insects were captured during the decay process. After the larvae migration, they were carried to the laboratory alongside the beneath soil for rearing procedure until the emergence of the adulthood that were then identified and their level of parasitoïdism estimate. During the whole experiment, 1976 individuals were sample with 121 captured in the field and 1855 emerged from the rearing in the laboratory. These flies belongs to 2 orders (Diptera and Coleoptera), 6 families [(Calliphoridae, Muscidae, Sarcophagidae, Tachinidae (parasitoïds), Dermestidae and Histeridae (Coleoptera)].

Keywords: Forensic entomology, parasitoïdism level, necrophagous flies, Cameroon

Introduction

The research of valuables proof is the main task of the judicial system worldwide. These proof must be as truth/real as possible in order to enable the justice to issue the correct sentence for a criminal case. Some of these elements are silent witnesses namely arthropods gathered on and around the death carcasses (Feugang Youmessi, 2023) [11]. For these invertebrate organism, this subtract offer also food, breeding site, opportune refuge (Amendt *et al*., 2004) [2]. Among necrophagous insects, literature (Frederickx *et al*., 2013; Rivers, 2016; Odo and Iloba, 2020; Pittner *et al*., 2020) [15, 30, 39, 46] always mentioned two orders Diptera and Coleoptera as widely present on decomposing animal carcasses. Necrophagous insects colonize the body immediately after deathwhen climatic conditions are favorable; they are also able to recognize the imminence of an animal's death and then, rush to natural orifices to lay their eggs, when no smell is yet perceptible to the human sense (Kelling *et al*., 2003; Feugang Youmessi *et al*., 2012 and 2021; Khoobdel and Davari, 2011 and Takusu *et al*., 2007) [22, 14, 12, 24, 34]. Goff (2010) [19] and Frederickx *et al*. (2014) [16] has shown that predators and parasitoïds are generally the second most important group frequently collected from cadavers. Among them, some parasitoïds live exclusively on the other arthropods they infest, mainly insects, eventually leading to their death (Amendt *et al*., 2010) [3]. During the decomposition process, large numbers of Diptera larvae and pupae attract a rich community of parasitoïds, including species belonging to the families Braconidae, Ichneumonidae, Pteromalidae, Figitidae, Eulophidae, Chalcididae, Diapriidae and Tachinidae (Voss *et al*., 2009) [36].

Knowledge of the diversity and the behavior of insect associated with carcasses can be used to estimate the post-mortem interval (PMI) or the time elapsed since the death of an animal (Anderson and Van Laerhoven, 1996) [4].

Several studies on forensic research focus mainly on nercophagous entomofauna (Amendt *et al*., 2010) [13] while the study of the attack of their life cycle witch affect the accuracy of PMI is neglect (Münster-Swendsen, 2002). Parasitoïds are the most dangerous pest of necrophagous insects since their activity can increase or reduce the PMI, as their attack time is often limited to a well-defined period in the development cycle of the host insect (Godfray, 1994; Amendt *et al*., 2010; Dheilly *et al*., 2015 and Heimpel and Mills, 2017) [18, 3, 9, 21].
This research work is part a program engages to estimate the level of parasitoïdism on necrophagous entomofauna in Cameroon in view of integrated it for the accuracy of post-mortem interval estimation as proof for legal purpose.

Materials and Methods

Study Site

Our experiment was carried out in a 2-year-old abandon farm located behind the third building of the Faculty of Science at 200m from the building (03°,85'94,8" N; W11, 49'92,5" E; altitude 756 m) in the campus of the University of Yaounde 1, during the period comprise from the 15th January to 16th February 2023.
Suchel (1988) [33] and Kengne Fodouop and Atangana (2010) [23] describe the climate of this area as “Yaounde-type” with four (04) distinct seasons of unequal duration: a long dry season from mid-November to mid-March, a short rainy season from mid-March to mid-June, a short dry season from mid-June to mid-August, and a long rainy season from mid-August to the end of October. The rainfall in Yaounde varied significantly over the year. The average annual rainfall fluctuates between 1,500 mm and 2,000 mm while the average annual temperature varied between 19 °C to 33 °C. The landscape of this part of the city is characterized by the presence of *Elaeis guineensis* (Arecaceae) and *Musa* sp. (Musaceae).

Experimental Procedure

The experiment was carried out on two rats carcasses (*Rattus norvegicus*, Berkenhout, 1769 var Wistar); these carcasses were placed inside a wooden cage measuring (1 m) x (1 m) x (1 m) x (1 m) with a 2 cm mesh between two consecutives pieces of wood to allow insects to enter while protect corpse from scavengers (Feugang Youmessi, 2014) [13]. Two complementary sampling methods were used during this research work for good estimation of the necro entomofauna of the area.

Killing the rats

In accordance with the rules governing the ethics and killing of animals for experimental purposes, the rats were brought to the site, tranquillized with carbon dioxide by its inhalation through vaporization by a veterinarian to avoid suffer to animal.

Field sampling of necrophagous insects

The rat corpses were observed immediately after being placed in the cage in order to record as accurately as possible the time and activity of the first insects to colonize the remains. As also emphasize by Wyss and Cherix (2006) [37], at their arrival, these insects first flew over the corpses for a while, probably to analyze, appreciate them and see if they are suitable for the development of their eggs. At the end of this exercise, which last a few minutes, they lay clusters of eggs in the natural orifices of the cadavers. The capture lasted for 20 minutes, then the net was vaporized with 70% alcohol to kill insect to make them to be easily pick with flexible forceps; they are then preserve in pillboxes containing 70% alcohol for further identification.
Sampling of the necroentomofauna was carried out three times a day from 8 a.m., at regular 4-hour intervals time periods to minimize disturbance of the carcasses colonization by necrophagous insects with our long time presence around the corpse during the first post-mortem two week, and once a day, at 12 p.m. during the rest of the cadaveric degradation period (Feugang Youmessi *et al*., 2021 and 2023) [12, 11].
Non-flying arthropodofauna were collected using flexible forceps, which allow insects to be caught without being crushed. During each visit to the field, the remains were meticulously observedand the fauna hidden beneath were sorted (Amendt *et al*., 2004) [2]. The overall necrophagous entomofauna preserved in 70% alcohol was brought back to the laboratory, stored for further identification.

Larval rearing

Once the migration of the larvae into the beneath soil contained in the box was observe, the carcasses were removed to place on new layer of sterilized soil containing in another box. The old box brought back to the laboratory for larvae rearing under natural ambient air temperature until the adulthood. According to Dao *et al*. (2018a) [43], the emerged flies were fed for 48 hours with cotton moisten with sugar water, then captured and stored in pillboxes containing 70% alcohol pending identifications.

Soil analysis

The soil analysis was perform in the laboratory of soil management of the Department of Soil and Univers Sciences of the University of Yaounde 1 according to the methods used by Robinson (Rouiller, 1993) [31].

Identification of insects

Insect specimens were identified under a vevor binocular loupe, using the identification keys of Delvare and Aberlenc (1989) [8], Kurahashi and Kirk-Spriggs (2006) [42], Wyss and Cherix (2006) [37], Feugang Youmessi *et al*. (2012) [14], Irish *et al*. (2014) [40] and Rochefort *et al*. (2015) [41].

Data analysis

The Excel workbook or spreadsheet version 2016 was used for data analysis such as the calculation of relative *Abundance* (F), which is the ratio of the number of individuals (ni) of a given taxon to the total number of individuals (N) of all taxa combinedthrough the formula F (%) = ni x 100/N (Zaime and Gautier, 1989) [38].
The Shannon-Weaver diversity index and equitability were used for better understanding of the data at the field and at the laboratory.

Results

Overall necroentomofauna captured during the experiment

During the entire experimental period, 1976 individuals composed all only of hexapoda were census with 1965 individuals belonging to the order Diptera representing 99.44%, and 11 specimens belonging to the order Coleoptera representing 0.56% of the whole necrophagous

entomofauna census along the experiment (table 1).
The order of Diptera was represented by 4 families (Calliphoridae, Muscidae, Sarcophagidae and Tachinidae) whereas the Coleoptera was comprised only of 2 families (Dermestidae and Histeridae). In addition to Diptera and Coleoptera species, the secondary taxa captured during the present research was Hymenoptera, family of Formicidae which will not be integrated in the following results.

Taxonomic composition and specific *Abundance*s of the necrophagous insect collected at the field

The field work enabled us to collect 121 individuals of adult flies belonging all to the class Hexapoda, two orders (Diptera and Coleoptera) and five families (Calliphoridae, Muscidae, Sarcophagidae, Dermestidae and Histéridae). Diptera were the most abundant with 116 individuals (95.9%) of the total compared to 5 insects of Coleoptera with 4.1%. The species *Abundance* was decreasingly made up of *Lucilia illustris* (Diptera), *Musca domestica* (Diptera), *Dermestes* sp. (Coleoptera), *Saprinus planiusculus* (Coleoptera) and *Sarcophaga* sp. (Diptera) with 101 insects (83.47%,), 14 individuals (11.56%), 4 insects (3.31%) and 1 insect (0.82%)respectively (table 2).

Taxonomic composition and specific *Abundance* of necrophagous entomofauna obtain from the larvae rearing in the laboratory

The rearing larvae within the laboratory yield 1855 specimens of hexapoda composed of the orders Diptera and Coleoptera respectively [1849 (99.7%) and 6 (0.3%)]. The most abundant taxa were Diptera with Calliphoriade identified as *Lucilia illustris* [1809 (97.5%)] and Tachinidae identified as *Dexia* sp. [40 (2.2%)]. The only Coleoptera emerged were *Saprinus planiusculus* (Histeridae) with 6 insects representing 0.3% of the emerged fauna from the laboratory (table 3).
This experiment enabled us to collect insects of the class of Hexapoda, including five (5) families from the field and three (3) in the laboratory. The combine interpretation of these flies has shown that *Lucilia illustris* and *Saprinus planiusculus* were both sampled in the field and in the laboratory, while *Sarcophaga* sp., *Dermestes* sp. and *Musca domestica* were only census at the field. Also, *Dexia* sp. (family Tachinidae) were only obtained from the insects emerged from the larvae rearing in the laboratory meaning that they are parasitoïd of larvae.
The substrate used for rearing in this work was a sterilized loan soil, i.e. rich in plant debris with a compact thick structure; its texture was 40.27% clay, 33.46% silt and 26.27% sand (table 4).

Data analysis

The data analysis is recapitulated inside the table 5.At the field, Shannon-Weaver diversity index (H') is lowed (0.5923) alongside the equitability (E) also small (0.368) at the field (table 5) showing the cohabitation amongst the whole field nercroentomo fauna that invade the carcasses during the decaying process except the Calliphoridae who were the majority in one hand and that the distribution of these specific insect isn't fair in the other hand probably the cause of poor low equitability value (0.368).
During the rearing of the migrated larvae at the laboratory, the above mention indexes weren't high (0.1258 and 0.1145 respectively) illustrating the poor specific diversity between the emerging necro entomofauna with the high number of Calliphoridae and also the unreasonable distribution of this entomofauna (table 5).

Discussion

During the experiment carried out from 15 January to 16 February 2023 (dry season), the overall fauna gathered was necrophagous consisted of 6 families grouped into two orders: Diptera with 4 families and Coleoptera hosted 2 families. The results are similar to those obtain on pig cadaver by Kpama-Yapo *et al*. (2021) [26] in the Guinean zone of Côte d'Ivoire, on grind beef meet by Fantio *et al*. (2022) [10] in Douala and on rat by Feugang Youmessi *et al*. (2021) [12] during their inventories of the necrophagous entomological fauna feed on decaying material.
These specific fauna act only few hours after exposure where numerous Diptera belonging to the Calliphoridae, Sarcophagidae and Muscidae families were captured. This early colonization of the corpse is the consequence of the inhalation of the odors issued by the cadaveric alteration process. Insects are known to have specific senses organ for detecting particular molecules or a family of chemical molecules within their antenna which act as peripheral micro-sensors for odorous in the environment (Charabidze *et al*., 2012; Kpama-Yapo *et al*., 2021) [6, 26].
Specimens from two Coleoptera families were present on the rat carcasses, namely Histeridae and Dermestidae, which corroborates the work of Dao *et al*. (2018a and 2018b) [43-45] on the succession pattern of necrophagous insects in Ivory Coast. Certain Coleoptera (Cleridae and Staphylinidae) regularly cited as necrophagous insects were absent from the rats remains; this absence is thought to be due to the harsh environmental conditions (temperature and relative humidity) during the experimental period (Kpama-Yapo *et al*., 2021) [26], and the anthropisation of the site (Wyss and Cherix, 2006) [37], since it has been cultivated for several years.
Our experience showed the predominance of Calliphoridae, followed by Muscidae, Tachinidae and Sarcophagidae. Specimens of the Tachinidae family represented here by the genus *Dexia*are often described as parasitoïds of the larvae of necrophagous insects (Stireman *et al*., 2006) [32] collected during this study. This predominance looks like the results of Feugang Youmessi *et al*. (2021) [12], Matuszewski*et al*. (2019) [44] and Pittner *et al*. (2020) [46] during their forensic research works.
Among these fauna, the parasitoïds obtained belong to the family Tachinidae which are known to be polyphagous meaning that they can be find infesting larvae during life cycle of necrophagous insects as emphasized by Stireman *et al*. (2006) [32]. However, the migration of the larvae within the beneath soil protect them fromparasitoïdism, confirming that they were infeste before their act of migration. This finding is also support by the granulometry of the type of soil used that shows a high percentage of clay (40.27%) which does not favour the insertion of the ovipositor of a parasitoïd to touch the larvae in view of eggs laying (Frederickx *et al*., 2013; 2014 and Feugang Youmessi, 2023) [15, 11].
The high *Abundance* of host rate suggests that there was a low infestation probably due to the drastic abiotic conditions of the study area since by the definition, parasitoïds generally kill their hosts at the end of their development cycles (Frederickx *et al*., 2014) [16]; therefore, if there were

more parasitoïds, there would be fewer hosts (Geden and Hogsette, 2006) [17]. The absence of these flies amongst the field collection was also noted by some researchers and can be explain by the fact that it is known that the odors issue by the carcasses seem to attract parasitoïd only when it is at a short distance i.e. 2 to 20 cm (Vinson, 1981; Takasu *et al*., 2007 and Frederickx *et al*., 2014) [35, 34, 16].

The biomass of the death corpse on which the larvae of necrophagous flies develop is ephemeral because it is continuously being eating by the larvae since their voracity is to high (Agriope, 2011) [1]. When these larvae are satiated, they migrate into the beneath soil to pupate (Gomes *et al*., 2006) [20] where some(Piophilidae) pupate close to their food source while others (Calliphoridae and Muscidae) leave the carcass and sunk deep into the soil to pupate (Voss *et al*., 2009) [36]. King and Ellison (2005) [25] and Reznik *et al*. (1992) [29] have shown that the deep migration of the host considerably reduces parasitoïdism. We collected mainly Callliphoridae and a few Muscidae during our experiment; these taxa are not vulnerable to parasitoïdism, as their larvae sunk deep into the soil for pupation (Voss *et al*., 2009) [36]. This results therefore corroborate those of King and Ellison (2005) [25] and Voss *et al*. (2009) [36]. Reznik *et al*. (1992) [29], Asgari and Rivers (2011) [5], Pitzer *et al*. (2011) [28] and Rivers (2016) [30], who also demonstrated that the burial of larvae in the soil was a prevention variable against parasitoïdism. These authors stated that certain larvae of the necroentomofauna such as those of Calliphoridae and Muscidae can bury themselves at a depth of more than 6 cm, which protects them against parasitoïds infestation of their pupae. With a low rate of emergence of parasitoïds *Dexia* sp. against a high emergence of Calliphoridae and Muscidae, this results are in line with those of the above mention authors.

This research work showed that the season may have an effect on the parasitoïds effectiveness. The evaporation during the long dry season hardens the soil and would not have facilitated burial of the larvae on the one hand and their parasitoïdism level on the other hand; this hardening of the soil (clay) would have limited the infestation of the larvae by the parasitoïds *Dexia* sp. These specific parasitoïds are nocturnally active and also not necrophagous so, after laying their eggs at night, they escaped. This strategy could help to explain their absence in the field samples and in addition to that behavior, the sampling times weren'tcorresponding to their egg-laying activity period (Stireman *et al*., 2006) [32].

Table 1: Overall necrophagous fauna gathered along the experiment at the field and from the rearing in the laboratory.

Ordrers	Families	Genus/Species
Diptera	Calliphoridae	*Lucilia illustris*
	Muscidae	*Musca domestica*
	Sarcophagidae	*Sarcophaga* sp.
	Tachinidae	*Dexia* sp.
Coleoptera	Dermestidae	*Dermestes* sp.
	Histeridae	*Saprinus planiuculus*

Table 2: Taxonomic composition and specific *Abundance*s of the necro entomofauna collected at the field.

Orders	Families	Species	Abundance (%)	Total number of individuals per order (%)
Diptera	Calliphoridae	*Lucilia illustris*	101 (83, 47)	116 (95,9)
	Muscidae	*Musca domestica*	14 (11, 56)	
	Sarcophagidae	*Sarcophaga* sp.	1 (0, 82)	
Coleoptera	Dermestidae	*Dermestes* sp.	4 (3, 31)	5 (4,1)
	Histéridae	*Saprinus planiusculus*	1 (0, 82)	

Table 3: Inventory and *Abundance* of necrophagous fauna in the laboratory

Orders	Families	Genus/Species	*Abundance* (%)	Total number of individuals per order (%)
Diptera	Calliphoridae	*Lucilia illustris*	1809 (97,5)	1849 (99,7)
	Tachinidae	*Dexia* sp.	40(2,2)	
Coleoptera	Histeridae	*Saprinus planiusculus*	6(0,3)	6 (0,3)

Table 4: Proportion of particles in the type of soil used

Variables	Clay	Silt	Sand
Granulometric proportion of loan soil	40.27%	33.46%	26.27%

Table 5: Shannon-Weaver diversity index (H') and Equitability index (E) at the field and within the laboratory

Parameters	Field	Laboratory
S	5	3
Number on insect	121	1855
H'	0.5923	0.1258
E	0.368	0.1145

Conclusion

During this experimental work, the entomological fauna census on rat carcasses was composed of Diptera and Coleoptera, (Calliphoridae, Muscidae, Sarcophagidae and Dermestidae), including scavengers predators (Histéridae, Formicidae) and parasitoïds (Tachinidae of the genus *Dexia*). In the laboratory, the emergence of the adulthood yields the taxa that didn't occurred amongst the field collection namely the family Tachinidae meaning that that group don't act during the sampling hours or has face difficulty to parasitized larvae probably due to the drastic abiotic parameters of the study site alongside the sampling period and the pedological parameters (clay for exemple).

The high *Abundance* of potential hosts in our experiment were obtained for Calliphoridae, Muscidae and few Sarcophagidae. After insect emergences, the level of the parasitoïdism of the necrophagous flies by *Dexia* sp. (Tachinidae), was only 2.2% emphasizing that the correlation between abiotic variables and season may affect the behavior of the parasitoïd on necrophagous entomofauna. The further work will deeply be concentrated on the issue.

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Conflict of interest: The authors declare that they have no conflict of interest.

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