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A preliminary investigation of insect colonization and succession on remains of rabbits treated with an organophosphate insecticide in El-Qalyubiya Governorate of Egypt

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ABSTRACT

A preliminary field study in the summer in a rural village in El-Qalyubiya Governorate (Egypt) compared the necrophagous insects colonizing the cadavers of two male rabbits (*Oryctolagus cunicullus domesticus* L.) killed by asphyxia (control), with two poisoned by the organophosphate (OP) pesticide pirimiphos-methyl (test). Decay of control carcasses was rapid since they reached the skeletal stage in only 19 days. Test carcasses did not decay completely, even 40 days post-killing. Insect species colonizing both carcasses types were not different, indicating that despite its odor, the OP were not masking the decomposition odors which were drawing the species to the bodies. The blowfly *Chrysomya albiceps* (Weidemann) constituted 76.6% of all samples collected. They were the first colonizers and played a major role in the decomposition process of control carcasses and in the partial decay of the test ones. They were significantly fewer numbers of immature stages developing on the test carcasses which probably contributed to their distinct lag and poor decomposition. Samples indicate that 17.3% of the insects were members of the Formicidae. This family was present in all carcasses, mostly in the early stages of decomposition. Formicidae may be considered omnivorous, and one of the fauna which use the cadaver as a refuge, to obtain humidity and food. This study provides additional knowledge in the context of Egyptian forensic entomology and the influence of OP which is of relevance to forensic science.

1. Introduction

Forensic entomology is the use of insects (and other arthropods) that inhabit decomposing remains to uncover circumstances of interest to the law and to aid in legal investigations [1]. It is based on understanding the arthropod sarcosaprophagous community. The usual classification of sarcosaprophagous fauna divides them into five distinct ecological groups: necrophagous, necrophilous, omnivorous, opportunists and accidentals. In general, necrophagous, necrophilous and omnivorous arthropods are the most important for forensic purposes [2]. Entomotoxicology is the application of toxicological analysis to carrion-feeding insects in order to identify drugs and toxins present on intoxicated tissues, and the effects caused by such substances on arthropod development. This assists in determining minimum post mortem interval (PMI) estimates [3–5].

In many parts of the world, mostly in the developing countries, the ease of availability of OP insecticides for agricultural purposes makes them one of the most important causes of poisoning by accidental exposure, suicide and sometimes, homicide [6]. El-Karadawy et al. [7] followed some acutely poisoned cases in Egypt, where 54% of them were poisoned by OP. Suicide attempts by intravenous injection with OP is an unusual way of intoxication which has been reported in some countries [8,9]. Pirimiphos-methyl is a cheap anticholinesterase OP pesticide that is widely used for agricultural purposes in the world and particularly in Africa to protect food against pests. Its acute and chronic toxicity had been well documented [10].

Forensic entomology and entomotoxicology are under investigated in Egypt except for a few cases [11,12]. The present study is intended primarily to establish a baseline data for the decay process and to document the faunal succession inhabiting decomposing rabbit carrions, placed in an inhabited area, in a rural village in El-Qalyubiya Governorate (Egypt) in the warm season. The second objective of this study was to compare these results with the corresponding data for decomposing rabbit carcasses, which were killed by OP-poisoning.

2. Materials and methods

This study was conducted in a rural village in Shebin El-Qanatir (30°19′N; 31°18′E; El-Qalyubiya Governorate), 35 km from Cairo, from the evening of the 20th

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Table 1
Rabbit carcasses decay duration and their associated arthropod fauna.

Stage of decomposition	Test		Control	
	Duration (days post-death)	Morphological change	Duration (days post-death)	Morphological change
Fresh	0–1	Discoloration in skin	0–1	Discoloration in skin
Bloat	1–3	More obvious body swelling and inflation than "control"	1–2	Gradual inflation then deflation Animal became more solid
Wet decomposition	4–10	Wandering only in the lower part. Fluid leakage from only the lower part. Irritating gases emitted from carcasses.	2–7	Wandering of skin Extensive fluid leakage
Dry decomposition	10–40	Reduction in flesh and fluid of the lower part. Abundance of dry constitutes of carcass: skin cartilage and bones, only in the lower part. Hardening of the upper part. No change appeared even after 40 days.	7–19	Reduction in flesh and fluid. Abundance of dry constitutes of carcass: skin cartilage and bones.
Skeletal			19–	Absence of soft tissues. Only bones, cartilage and hair remains

August to 30th September 2008. Because they are small and easy to obtain, and to allow the concentration of OP used, the experiment was performed on four male rabbits (*Oryctolagus cunicullus domesticus* L.), each weighting approximately 1500 g. Two control rabbits were killed by asphyxia. The other two test rabbits were poison killed by the administration of 10 ml pure dose of an anticholinesterase OP pesticide directly injected into the apex of the heart: death followed immediately. The pesticide was Norish® (pirimiphos-methyl 50%, Sorbol 10%, odorless kerosene 40%) is an anticholinesterase OP compound. It was supplied in a liquid form from El-Fares Company, under registration # 757. This pesticide is widely used in rural villages in Egypt for eliminating walking pests (cockroaches, ants, etc.).

Within 10 min of death, the four carcasses were transferred into four separate cages welded with wire mesh (1 cm), to exclude vertebrate scavengers. They were then placed 1 m apart in an uninhabited house. The day of killing and placement of rabbit carcasses was designated as day 0.

The temperature ranged from 32 to 36 °C, with an average daily maximum temperature of 34.1 °C and minimum temperature of 23 °C. The mean relative humidity averaged about 38% during the study period. The cages were visited daily at mid-day for the first two weeks and then once a week for the following four weeks. During each visit, insect activity was observed and representative samples of adult and immature stages collected from, on, in, around and beneath the carcasses. Sampling of the specimens was opportunistic (non-random sampling) by sweep net, forceps and spoons (for the larvae masses). Approximately 25% of specimens were captured from each case. Adult live specimens were killed and placed into a vial which is then put into a freezer (−20 °C). Mass larvae were immersed in near-boiling water for 30 s and then transferred into 75% alcohol (according to the opinion of the board and the members of the European Association for Forensic Entomology [13]). Dead samples (if any) were also removed and maintained in a domestic freezer (−20 °C). A small number of live Dipterous larvae and puparia were reared in small vials (larvae were fed rotten minced meat) at room temperature for confirmatory identification purposes. The arthropods collected were identified at the Museum of Entomology Department, Faculty of Science, Ain Shams University, Abbassya, Cairo. Identifications of the adult Diptera were made using Steyskal [14] Shaumar and Mohammad [15] and Shaumar et al. [16]; of Hymenoptera using Mohammad et al. [17] and of Coleopteran species using Shaumar et al. [18] and Essam [19]. SPSS v.11.0 software was used to analyze the data.

3. Results

Five observable stages of decomposition were recognized in the control carcasses (Table 1). Their decay was rapid; 19 days post-killing, these remains reached the skeletal stage, defined by complete loss of soft tissues with only bones, cartilage and hair remains present. In contrast, there was a distinct delay in the decomposition process of test carcasses relative to the control. Test carcasses had undergone only partial decay even 40 days following OP poisoning with only the lower parts of the test carcasses obviously decayed, whilst their upper parts remained unchanged.

Insects played a significant role in both cases. Carcasses yielded 16 arthropod species representing five orders and 10 families (Table 2). Calliphoridae represented 76.6% of all samples collected from both groups. They were the first colonizers and played a major role in carcasses decay. Other well-represented groups were Formicidae (17.3%), Dermestidae (3.3%) and Histeridae (2.1%); other taxa amounted to less than 1%.

The trophic relationships among the species are shown in Fig. 1. Interestingly, the insect species colonizing both types of carcasses

Table 2
Arthropods associated with rabbit carcasses (killed through intoxication by organophosphate and an untreated control) in Shebein El-Qanater (El Qalyubiya Governorate, Egypt) in Summer 2008.

Class	Order	Family	Genus/species	Test (%)	Control (%)
Insecta	Diptera	Muscidae	*Musca domestica* (Linnaeus)[a]	0.9	0.2
			Stomoxys calcitranus(Linnaeus)	0.1	0.1
		Calliphoridae	*Chrysomya albiceps* (Weidemann)[b,a]	57.1	81.5
			Chrysomya marginalis (Wiedeman)[b]	0.2	–
			Calliphora vicina Robineau-Desvoidy[b]	0.4	0.2
		Sarcophagidae	*Parasarcophaga aegyptiaca*(Salem)	0.3	–
	Coleoptera	Dermestidae	*Dermestes maculatus* (DeGeer)[b,a]	6.8	2.3
		Histeridae	*Saprinus bonnairei* Fairmaire	2.0	0.4
			Saprinus chalcites Illiger	3.7	0.2
			Saprinus semipunctatus Fabricius	1.0	0.2
		Tenebrionidae	*Ocnera andresi* Gridelli	0.3	0.1
		Cleridae	*Necrobia rufipes* (De-Geer)	0.2	–
		Undefined	Undefined larva	–	0.1
	Hymenoptera	Formicidae	*Monomorium sp.*	26.8	14.6
	Neuroptera	Myrmeleontidae	*Myrmeleon* sp.[c]	–	0.1
Arachnidae	Arachnidae	Undefined	Undefined	0.2	0.1
Total[c]				100	100

[a] Identified with their larvae also.
[b] Species with primary forensic importance.
[c] Pearson Chi-Square test for test of proportions ($P = 0.000$).