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Original Research Article

IDENTIFICATION OF FORENSICALLY IMPORTANT INSECTS ON ATRAZINE-INTOXICATED RAT CARCASSES AT DIFFERENT DECOMPOSITION STAGES DURING SUMMER SEASON

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Abstract: This study was designed to assess the effect of atrazine, the most commonly used herbicide in Egypt, on the decay process of intoxicated rat carrions and their associated forensically important insects during the summer season. Six Sprague-Dawley rats were assigned into groups. Cervical dislocation was used for killing the 1st group, and served as the control, whereas atrazine intoxication was utilized to kill the 2nd group. Control carcasses decomposed quickly, taking only 19 days to reach the skeletal stage. Decay of atrazine-intoxicated carrions was delayed since they reached the skeletal stage in 30 days. In addition, a delay in the colonization of insect fauna for atrazine-intoxicated carcasses was observed. The predominant necrophagous arthropods involved in the decomposition process were the orders Diptera and Coleoptera. The insect family Calliphoridae was the first to colonize the corpses and persisted until the beginning of the advanced decay stage. The results revealed that atrazine affected the succession pattern of carrion-feeding insects. In the control group, Dipteran insects were the most dominant insects (57.14%), and then Coleopteran insects were the following ones (42.85%). While the atrazine-intoxicated group showed 42.85% for insects of order Diptera and 57.14% for Coleoptera. This work suggested that the atrazine intoxication affected the decomposition process of rat carrions and their related arthropod fauna during the summer season, which could aid in the future forensic investigations of suspected pesticide poisoning.

Key words: forensic entomology; decomposition; entomotoxicology; insect succession; atrazine

Introduction

Forensic entomology is a new discipline of forensic science that investigates the biological and ecological characteristics of colonizing arthropods and insects (1). It could provide scientific information on the succession pattern of the insects with forensic significance. This branch of science is highly valuable in the determination of postmortem interval (PMI) via morphological identification and estimating the developmental stages of such insects detected on the corpse (2).

Following death, a variety of natural changes occur in the carrions as a result of the actions of microorganisms and arthropods that use the carrions as a changing and transitory source of food (3). Arthropods arrive and inhabit human and animal carrions in an expected and distinct manner in the absence of vertebrate scavengers, and they play a vital role in carrion decomposition in association with anaerobic microorganisms (4, 5). Diptera and Coleoptera are the most significant orders in the decomposition process because their immature stages feed on carrion (6).

The decomposition process, faunal constitution and succession, and insect developmental time are affected by certain factors, including

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temperature, rainfall, wind, and geographical locality. Therefore, when local climatological data is available, the colonizing fauna sequence could be used to determine the PMI (7).

Entomotoxicology is the use of toxicological examinations on insects that feed on corpses to detect the toxins and drugs found in the affected tissues, as well as the consequences of these compounds on the arthropod development (8). Furthermore, recent reports have shown that the presence of drugs or toxins in decayed tissues can affect the pattern and rate of colonization of insects (9-11).

Atrazine is a broad-spectrum herbicide, which is used extensively in agriculture worldwide (12). It is found in the environment as a ground and surface water contaminant in addition to industrial sources (13). The detection of atrazine in ecosystems and humans has been documented due to its mobility and long-term environmental persistence. Atrazine is known as an environmental endocrine disruptor, provoking its toxic effects on various target organs in the body such as liver, kidney, testis, and brain through induction of oxidative stress (12, 14, 15). To our knowledge, research in the disciplines of entomotoxicology and forensic entomology is still scarce in Egypt, excluding a few publications (9-11, 16, 17). Thereby, the 1st aim of this work is to set background information about the decomposition process and to record the insects of forensic importance inhabiting decaying rat carrions in Zagazig, Sharkia Governorate, Egypt during the summer season. The 2nd goal of the present work is to perform a comparison between these results and those of the decomposed rat corpses intoxicated with atrazine.

Materials and methods

Chemicals

Gesaprim (Atrazine 80% wettable powder) was purchased from Syngenta CO., Bazal, Switzerland. The chemical formula of Atrazine is 6-chloro-4-N-ethyl-2-N-propan-2-yl-1, 3, 5-triazine-2,4-diamine; whereas its empirical formula is $C_8H_{14}ClN_5$.

Site and circumstances of the study

This investigation was performed in the Forensic Medicine and Toxicology Department and Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt. The buildings of the Faculty are situated in El-Zeraaha Square, Zagazig, Sharkia Governorate, Egypt, with longitude: 31°30'07"E and latitude: 30°35'15"N. This study was carried out in a room with a surface area of 24 m^2 (8m length × 3m width) at the Parasitology Department in the third floor (eight meters above the ground). In this room, a window and a door were closed, except for a 2 cm gap between the floor of the room and the bottom of the door, which allowed insects to enter. This site was ideal for monitoring of cadavers every day and avoiding interruptions caused by scavengers or animals, and it was marked with instructions to limit human intervention. The experiment was conducted in the summer season (July-September, 2019). During the experimental period, the weather was dry with moderately stable temperatures ranging from 22-36°C (29°C on average) and the relative humidity ranged from 49 - 57%.

Experimental animals and procedures

Six healthy male Sprague Dawley rats weighing 200 ± 20 g, obtained from the Farm of Laboratory Animals, Faculty of Veterinary Medicine, Zagazig University, Egypt, were allocated for the present study. The animals were divided into two equal groups (3 rats/each group). Rats were anesthetized with diethyl ether prior to the killing. Cervical dislocation (control group), and atrazine intoxication (atrazine -intoxicated group) were used to kill the rats. Cervical dislocation had performed via dragging rat's tails subsequent to neck compressing by a harsh object. Atrazine intoxication was done by single oral administration of rats with atrazine dissolved in distilled water at a dose of 6000 mg/kg b.wt (2XLD50). The LD50 of atrazine in rats was recorded to be 3000 mg/kg b.wt according to Hauswirth et al (18).

Rats were transported to the study room immediately after death (within 5 min) in a wire mesh-coated plastic cage of 50 cm long, 30 cm wide, and 30 cm high. Day 0 was designated as the day of killing and placing the corpses. The corpses had checked at midday every day for the 1st three weeks and after that twice weekly till rat remains had undergone full dryness without any active and live insect fauna detected inside the cages. The decomposition stages had been carefully registered and Canon digital camera was used to photograph the decomposition of the re-

mains and the detected insects.The Ethics of Animal Use in Research Committee of Zagazig University; Egypt had approved the current experimental procedures.

Sample collection and identification

The activity of insects was observed throughout the monitoring period, and some immature and mature insect samples were obtained from the carcasses (on, in, around, and below) (17).

Maggots were allocated into 2 groups. The first group was immediately killed by submerging them in boiling water for 30 sec, and then preserved in 70% ethanol with the addition of one drop of glycerin in case of long time preservation of larvae or preserved in 10% formalin for morphological examination (19), while the second group was kept for rearing in the laboratory.

Larvae rearing

The obtained larvae had been raised to complete the life cycle and obtain adult flies using the method adopted by John et al. (20). The flies, larvae, and pupa that had been collected and reared were identified following the keys of Bowman (21).

Results

The manner of corpse decomposition

The decomposition manner was identical in control and atrazine-intoxicated cadavers that included five marked stages; fresh, bloated, active decay, advanced decay, and lastly skeletal stage. The start and period for each stage as well as postmortem alterations in corpses of control and atrazine-intoxicated groups were described in Table (1). The postmortem findings for each decomposition stage in the control and atrazine-intoxicated carcasses were illustrated in Figure1.

Insect diversity

There were 2 orders, 8 families, and 10 species of insect fauna obtained from the carcasses of control and atrazine-intoxicated groups (Table 2). The predominant necrophagous arthropods, that promoted carcass decomposition, were belonged to the Coleoptera and Diptera orders. The insect family Calliphoridae was the first to colonize the carcasses of various groups, and it persisted till the commencement of the advanced decay stage.

Forensic insect abundance

The proportions of major orders detected on rat carrions in each group and between various studied groups were shown in Figure 2. The order Diptera insects dominated the insects in control corpses, as a whole (57.14%) followed by Coleoptera (42.85%). In contrast, the percentages of Diptera and Coleoptera were 42.85% and 57.14% in atrazine-intoxicated carcasses, respectively.

Insect succession pattern on rat carrion

The insect succession order of the control and atrazine-intoxicated rat carrions was represented in Figure 3

Fresh stage

This stage lasted for one day in the control and atrazine-intoxicated groups. All corpses revealed skin discoloration (Table1). Dipteran mainly Calliphoridae adult flies (*Chrysomya albiceps* and *Chrysomya bezziana* adult) were the major insects detected on the control rat carrions (Table 2). On the other hand, atrazine-intoxicated carcasses showed the absence of insect fauna in this stage.

Bloating stage

The bloating stage appeared on the 2nd day of killing in the control and atrazine-intoxicated rat carcasses and persisted for one day prior to the beginning of the active decay stage (Table1). Necrophagous arthropods at this stage primarily belonged to Dipteran families (Sacrophagidae and Calliphoridae) were the first to colonize all corpses in different experimental groups. These insects were noticed on control corpses (on the first day) earlier than the atrazine-intoxicated group (on the 2nd day). Adult Chrysomya *albiceps* and *Chrysomya bezziana* insects were the predominant species on all corpses and they played a major role in the decomposition process. Adult Chrysomya *albiceps*, *Chrysomya bezziana*, Wholfahrtia *magnifica*, and *Sarcophaga haemorrhoidalis* were detected on the carcasses of control and atrazine-intoxicated groups. While adult *Fannia canicularis* was detected only on control cadavers. However, *Necrobia rufipes* adult was detected only on the atrazine-intoxicated corpses on the 2nd day of the bloating stage (Table 2).

Table 1: Beginning, period and postmortem alterations of various decomposition stages in rat carcasses intoxicated with atrazine and control carcasses.

Stage of decomposition	Control		Atrazine intoxication	
	Start and period (days)	**Postmortem alterations**	**Start and period (days)**	**Postmortem alterations**
Fresh stage	0-1	Corpse revealed complete intact body with no physical changes. Only skin discoloration was detected. There were no visible morphological alterations, swelling or putrid odors.	0-1	Discoloration of skin No swelling. No putrid odors
Bloating stage	1-2	Swelling of carcasses. Odor of putrefaction became obvious. Decomposition fluids and exudates were noticed below the carrion. The corpse deflated at the end of this stage.	1-2	Swelling and inflation of the carcass were more visible than control. Putrefaction odor was more potent than control
Active decay stage	3-8	Complete decomposition skin wandering Extensive leakage of fluids Strong putrefaction odors. Hair loss particularly in areas with massive maggot's activity.	3-15	Wandering of skin was only observed in the lower part Fluid leakage from only the lower part Irritating gases emitted from corpses. Putrefaction odor was stronger than control Extensive leakage of fluids
Advanced (dry) decay	9-16	The majority of the fleshy tissue had disappeared Putrefaction odors were less pronounced Dry skin began to detach from the body.	16-30	Less fluid, flesh and, putrefaction odors and only dry tissues were present (skin and bones). Reduction in the flesh and fluids 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
Skeletal stage	16	Only dry skin, cartilages, and bones were noticed, along with a few scattered masses of remnant hair.	30	Dry skin, cartilages, bones, and hair remained

Active decay stage

This stage developed following two days in all carrions of the control and atrazine-intoxicated groups. The active decay stage persisted for five days in the control group, whereas it lasted for a prolonged period of time and continued for 12 days in the atrazine-intoxicated group (Table 1).

It was noticed that the active decay stage in the control and atrazine-intoxicated carcasses was promoted by the Calliphoridae larvae of Diptera order (mostly *Chrysomya bezziana* and *Chrysomya albiceps* larvae) that were found at corpses on the 1st day of this stage. Chrysoma pupa was detected in the control and atrazine-intoxicated corpses on the 4th day and 6th day of the active decay stage, respectively. Furthermore, *Wholfahrtia magnifica* larvae were detected on the carrions of atrazine-intoxicated group (on the first day) earlier than the control group (on the 2nd day). *Sarcophaga haemorrhoidalis* larvae were detected on the control and atrazine-intoxicated carcasses on the 2nd day of the active decay stage.

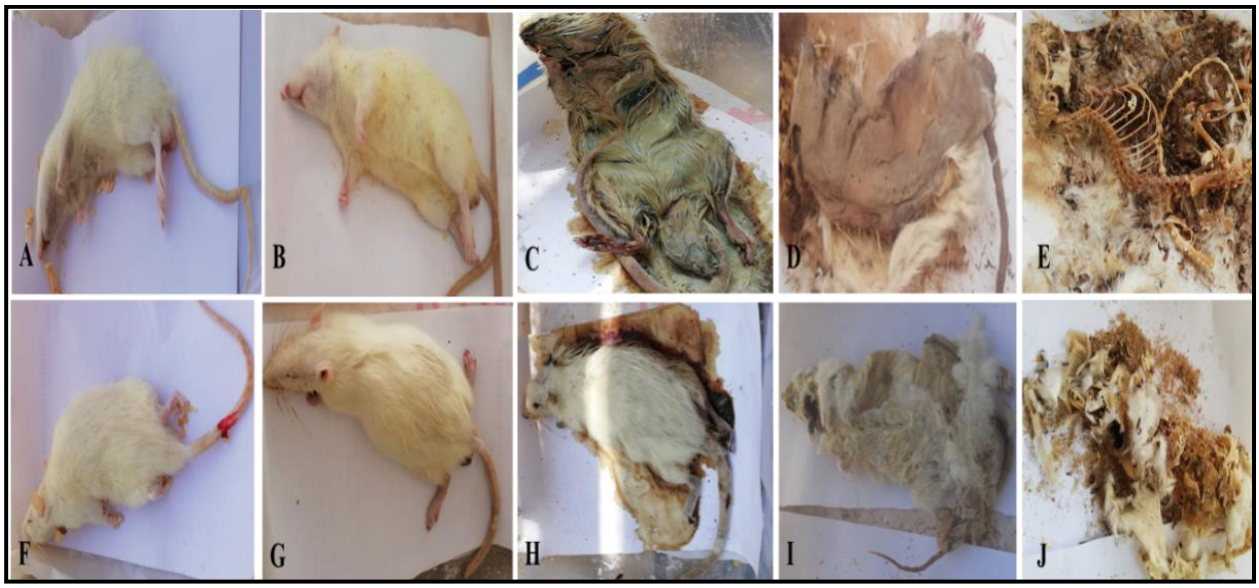

Figure 1: Stages of Decomposition in rat carrions from control group (A-E) and atrazine-intoxicated group (F-J). A; Fresh stage, B; Bloated stage, C; Active decay stage, D; Dry decay stage, and E; Skeletal stage. F; Fresh stage, G; Bloated stage, H; Active decay stage, I; Dry decay stage, and J; Skeletal stage

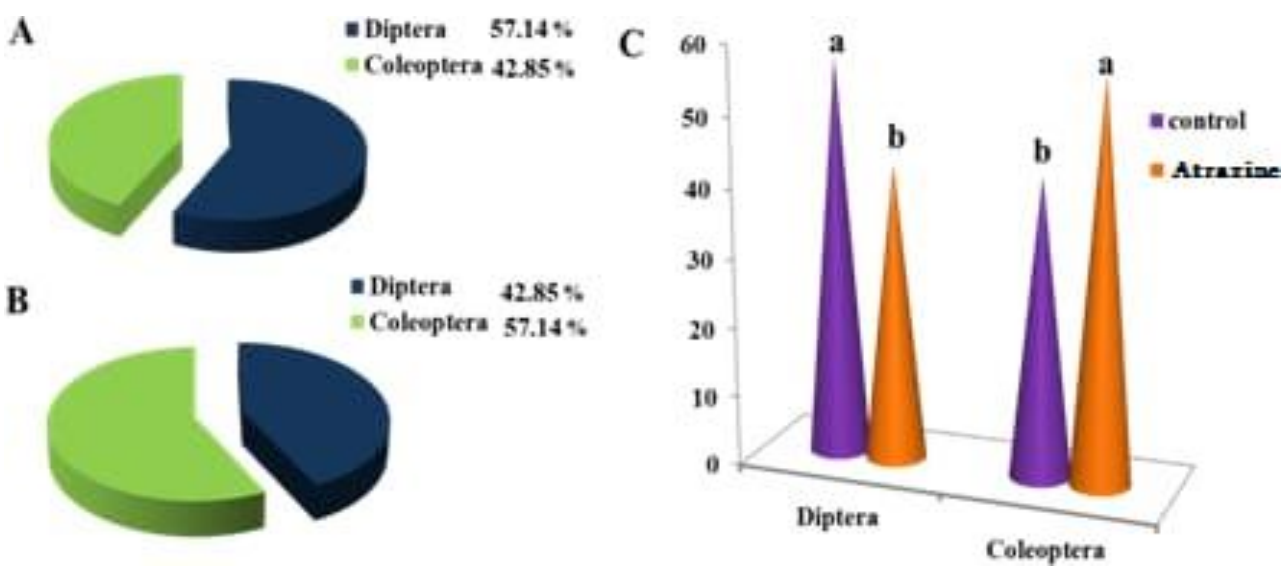

Figure 2: The percentages of the major orders of necrophagous arthropods detected on rat cadavers in each group. A; Control, B; Atrazine-intoxicated group, and C; The percentages of the main orders between two studied groups

The Sarcophagidae pupas (*Sarcophaga haemorrhoidalis*, and *Wholfahrtia magnifica*) were only detected in the control corpses on the fourth and fifth days, correspondingly. While the adult of *Wholfahrtia magnifica* was found only on the control carrions on the 4th day of the active decay stage.

Fannidae and Piophilidae adults (Fannia canicularis, *Piophila casei)* were observed only on the atrazine-intoxicated carcasses on the 3rd day and 11th day of this stage, correspondingly.

The 1st detected Coleoptera at the active dry stage were the adult of *Necrobia rufipes* and *Saprinus chalcites* that observed on the corpses of atrazine-intoxicated group on the 1st day. They were observed in the control group on the 2nd and 3rd day of this stage, respectively. The *Necrobia rufipes* larvae were detected in control and atrazine-intoxicated corpses on the 5th day.

Table 2: Diversity , and succession day of insects on rat corpses of control and intoxicated with atrazine at different stages of decomposition.

.Stage	Insects			Groups			
				Control		Atrazine intoxication	
	Order	Family	Species	*St	SD	*St	SD
Fresh stage	Diptera	Calliphoridae	*Chrysomya bezziana*	A	0	-	-
			Chrysomya albiceps	A	0	-	-
Bloating stage	Diptera	Calliphoridae	*Chrysomya bezziana*	A	1	A	2
			Chrysomya albiceps	A	1	A	2
		Sarcophagidae	*Wholfahrtia magnifica*	A	1	A	2
			Sarcophaga haemorrhoidalis	A	1	A	2
		Fannidae	*Fannia canicularis*	A	1	-	-
	Coleoptera	Cleridae	*Necrobia rufipes*	-	-	A	2
Active decay stage	Diptera	Calliphoridae	*Chrysomya bezziana*	L	1st-4th	L	1st-5th
			Chrysomya albiceps	L	1st-4th	L	1st-5th
			Chrysomya	P	4th	P	6th
		Sarcophagidae	*Wholfahrtia magnifica*	L	2nd	L	1st-5th
				P	4th	-	-
				A	4th	-	-
			Sarcophaga haemorrhoidalis	L	2nd	L	2nd-5th
				P	5th	-	-
		Fannidae	*Fannia canicularis*	-	-	A	3rd
		Piophilidae	*Piophila casei*	-	-	A	11th
	Coleoptera	Cleridae	*Necrobia rufipes*	A	2nd-4th	A	1st-6th
				L	5th	L	5th-15th
		Dermastidae	*Dermestes maculates*	A	2nd -4th	A	6th-9th
				-	-	L	7th-15th
		Histeridae	*Saprinus chalcites*	A	3rd	A	1st-6th
Advanced (dry) decay stage	Diptera	Calliphoridae	*Chrysomya*	P	3rd	-	-
				-	-	-	-
		Sarcophagidae	*Wholfahrtia magnifica*	-	-	P	1st
			Sarcophaga haemorrhoidalis	-	-	P	2nd
		Piophilidae	*Piophila casei*	A	1st	-	-
	Coleoptera	Cleridae	*Necrobia rufipes*	-	-	L	5th-15th
		Dermastidae	*Dermestes maculates*	L	1st -8th	L	5th-15th
				A	3rd-6th		
Skeletal stage	Coleoptera	Cleridae	*Necrobia rufipes*	L	21st	L	18th
				-	-	A	18th
		Dermastidae	*Dermestes maculates*	L	1st-21st	L	18th
				A	21st	A	18th
		Histeridae	*Hister sp*	A	16th	-	-

St; Stage of development, SD; Succession day
A; Adult, L; Larva, P; Pupa

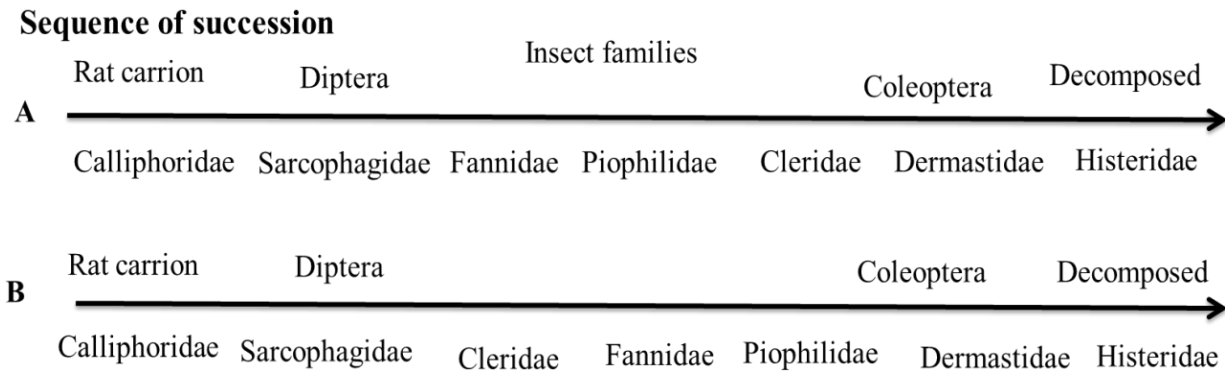

Figure 3: Insect succession order on the rat corpses. Control group (A), and atrazine-intoxicated group (B)

Dermestes maculates adult was noticed first on the control carcasses (on the 2nd day) then on the 6th day in the atrazine-intoxicated group. Meanwhile, *Dermestes maculates* larvae were detected only on the atrazine-intoxicated corpses on the 7th day of this stage (Table 2).

Dry decay stage

As illustrated in Table 1, the start and period of the advanced decay stage differed in the control and atrazine-intoxicated carcasses. The rapid onset and the shortest duration were noticed in the control group. The decomposition of the atrazine-intoxicated corpses was markedly delayed compared to the control ones. Even 40 days after atrazine intoxication, these corpses had only partially decomposed, with just the lower portions of the carrions clearly decayed, while the upper portions stayed unaffected.

The pupa of Chrysomya appeared only on the control corpses on the third day. Sarcophagidae (*Sarcophaga haemorrhoidalis* and *Wholfahrtia magnifica*) pupas were detected only on the atrazine-intoxicated carrions on the first day and 2nd day of the advanced decay stage, respectively.

Adult *Piophila casei* was found only on control corpses on the 1st day of this stage. *Necrobia rufipes* larvae had been detected only on the atrazine-intoxicated corpses on the 5th day of advanced decay stage.

The larvae of *Dermestes maculates* had been observed on control cadavers on the 1st day till the 8th day and on the atrazine-intoxicated carrions on the 5th day to the 15th day. *Dermestes maculates* adult was detected only on the control groups on the 3rd till 6th day of this stage.

Skeletal stage

The data in Table1 showed the variation in the onset of the skeletal stage in the control and atrazine-intoxicated groups. The skeletonization process started earlier in the control group (on the 16th day) than in atrazine-intoxicated carcasses (on the 30th day).

The dominant insect fauna in this stage was mostly Coleopteran. The larvae of *Necrobia rufipes* had been detected on control and atrazine-intoxicated corpses on the 21st and 18th day of skeletonization, respectively. Adult *Necrobia rufipes* was detected on atrazine-intoxicated corpses only on the18th day of the skeletal stage.

Dermastidae larvae (*Dermestes maculates*) had been detected on control carcasses on the 1st day of skeletonization until the day 21st. On the other hand, *Dermestes maculates* larvae had been detected on atrazine-intoxicated copses on the 18th day of this stage. *Dermestes maculates* adult was detected on the control and atrazine-intoxicated corpses on the 21st and 18th days, correspondingly. *Hister* species adult was detected only on control cadavers on the 16th day of skeletonization (Table 2).

Morphological identification of the observed insects

The observed insects were morphologically identified as previously described by Farag et al. (17).

Flies belonging to order Diptera
Family Calliporidae, Genus Chrysomya
Chrysomya bezziana

The adult has a metallic-green or blue body, orange-brown eyes and a length of 8-12 mm. The posterior borders of abdominal segments have

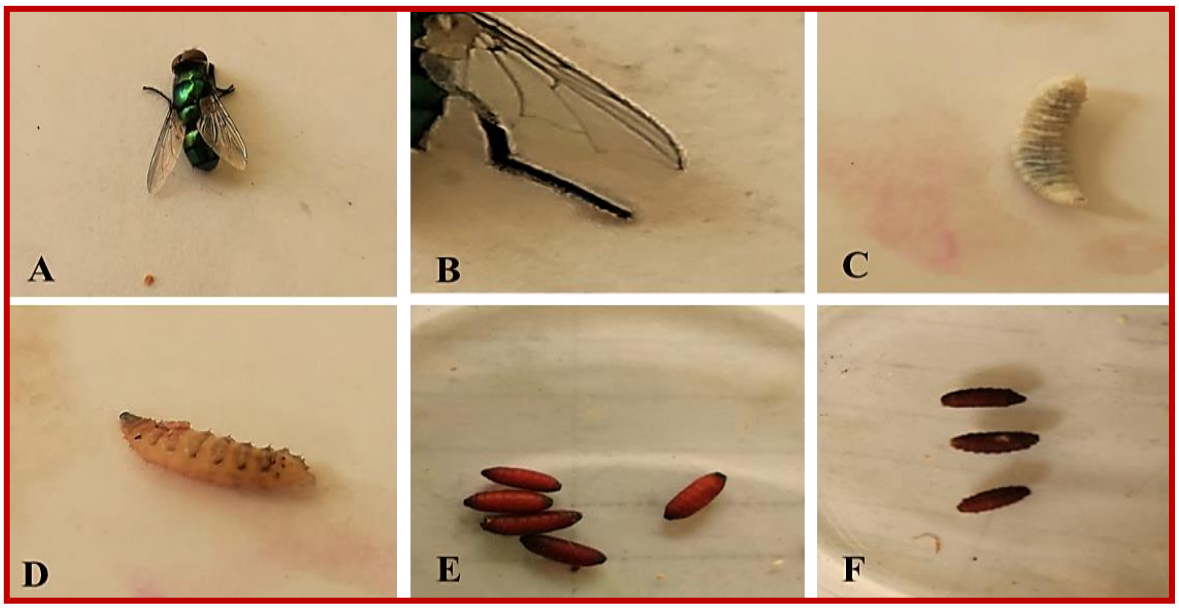

Figure 4: A; *Chrysomya* species adult, B; *Chrysomya albiceps* wing (X5), C; *Chrysomya bezziana* 3rd larval stage, D; *Chrysomya albiceps* 3rd larval stage, E; *Chrysomya bezziana* pupa, and F;

small dark stripes. A frontal stripe runs parallel to the female's head. The base of the wing is slightly darker than the rest of the wing. The fly has dark legs. Mature third stage larvae are nearly eighteen mm in length and creamy white (Figure 4).

Chrysomya albiceps

The adult is bluish-green and has orange-brown eyes. This insect is measured approximately 5-10 mm in length. The posterior borders of abdominal segments contain black strips. The thorax does not have a distinct pattern. The legs range in color from reddish brown to black. Fine hairs cover the lower squamae. Mature third stage larvae can grow up to eighteen millimeters in length and range in color from white to dark yellow. There are several fleshy protrusions resemble thorns on majority of their body segments, giving this species its common name (hairy maggot blow flies) (Figure 4).

Chrysomya albiceps pupa
Family Sarcophagidae
Wohlfahrtia magnifica (spotted flesh fly)

The length of an adult is approximately 11-14 millimeters. There are three dark stripes running along the thorax, and three dark, distinctive and circular patches on the abdomen. The larva is measured approximately 10-12 mm in length (Figure 5).

Sarcophaga haemorroidalis

The adult is measured about 11-15 mm long. The thorax has three dark black longitudinal stripes, whereas the abdomen has a chessboard pattern of grey and black patches. The larva ranges in length from 11to13 mm (Figure 5).

Family Fannidae
Fannia canicularis (Lesser house fly)

This insect is dark grey in color and has a yellow abdomen. Its thorax contains 3 black bands. It is about 4-6 mm long. There are simple wing veins on the wing, including a straight fourth longitudinal vein and a short sixth longitudinal vein. The 4th longitudinal vein is not curved as in *Musca* species.

Family Piophilidae
Piophila casei

is a small black fly and ranges in length from 2.5 to 4 mm. The cheese skipper is a common name for this species. They have yellow legs and antennae on the jowls of their faces. There are small and widely spaced ocellar bristles on the opposite side of the simple eye (front ocellus) (Figure 5).

Figure 5: A *Wohlfahrtia magnifica* adult, B *Sarcophaga haemorroidalis* adult, C *Piophila casei* adult

Figure 6: A *Dermestes maculatus* adult, B *Necrobia rufipes* adult, C *Hister* species adult

Beetles belong to the order Coleoptera

Coloptera beetles have biting mouthparts (mandibles) and eleven-segmented antennae. Though there may be less than 11 segments in some species. Prothorax (the first thoracic segment) is frequently characteristic in shape and size and could be served as a mean for the beetle identification. The exoskeleton of beetles is consists of tough plates.

Dermestes maculatus (the hide beetle)

The adult is generally oval and ranges from 5.5 to 10.0 mm in size. A band of white hairs runs along each side of the thorax. The abdomen's underside is mostly white with black spots on the sides. A big black patch is located on the last abdominal segment. Black, yellow, or white hairs cover the forewings (elytra), which are dark brown to black in color. They have short and segmented antennae that have a club at the end of them. On the abdominal end of the elytra, there is a tiny spine that protrudes straight out from the serrated edge of the elytra.

The larval body is coated in hairs with various lengths, named setae (woolly bears), which are arranged in rows. The abdomen's underside is usually yellowish-brown in color, whereas the dorsal surface is generally dark brown with a yellow line running down the middle (Figure 6).

Necrobia rufipes adult

The adult is about 3.9-7 mm in length. They contain 3 distinct body regions and are elongated oval in shape. They are blackish-blue in color with red legs. The antennae are eleven-segmented with an extended club-like area at the tip. Each wing has nine rows of tiny hairs covering it. Larvae are 8-10 mm in length. In the center of their bodies, they have three pairs of legs. The body is reddish to blackish in color, while their legs are pale mustard in color (Figure 6).

Saprinus chalcites adult

They are approximately 4-6.8 mm in length. The body is shiny, oval, and weakly convex. Pronotum is greenish-black in color, whereas elytra might be dark blue.

Hister species (Clown beetles)

They have a flat shape. The head contains two antennae and compound eyes that can sunken into the prothorax. The main distinctive mark is that the antennae are curved inwards to form a club at the tip. Elytra are usually lustrous black or metallic green in color. A pair of the seven tergites is usually exposed on each of elytra, which is shorter than the abdomen (Figure 6).

Discussion

The current work describes a brief investigation into the response of insect fauna, which has direct forensic implication to rat cadavers intoxicated with atrazine as it is a commonly used herbicide in agriculture in Egypt (22). The decomposition process of cadavers represents a series of well-ordered biological events that cause organic matter breakdown (23). This process takes place in five sequential stages. There are several factors that influence the duration of each phase, including biotic (i.e. insects and bacteria) and a biotic (i.e. weather conditions) factors, or the presence of toxins (9, 10, 24). In the current investigation, five distinct decomposition stages involving fresh, bloated, active decay, advanced decay, and lastly skeletal stage were observed in control and atrazine-intoxicated groups. Similar findings have been recorded in rat carrions in Egypt (17, 25) and other countries such as Iran (26). In contrast, Jales et al. (11) observed only 4 decomposition stages (fresh, bloating, active decay, and dry decay) on the rat carcasses intoxicated with Terbufos (Organophosphate).

In the current study, the fresh stage on the rat carcasses lasted for one day. In a similar study, the fresh stage took one day on organophosphate-intoxicated rabbit carcasses in El-Qalyubiya Governorate, Egypt (16). However, the fresh stage lasted only 0.05 day on the rabbit in Egypt, as recorded by Zeariya et al. (27). n Indian urban areas, Bharti et al. (28) reported 1.5 days for the fresh stage on rabbit cadavers. Abd El-Gawad et a.l (10) recorded that the fresh stage on the rabbit corpses lasted for two days in Cairo, Egypt.

Herein, the bloating stage continued for one day on the control and atrazine-intoxicated rat carcasses, whereas this stage lasted for 1-2 days in an earlier investigation performed in Egypt (27). Abd El-bar et al. (16) stated that the bloating stage lasted for 2 days on the rabbit carrions intoxicated with an organophosphate insecticide (pirimiphosmethyl) in Egypt. The variations in duration of fresh and bloating stages in this work compared to others can be attributable to the fact that the cadavers were kept indoor and not exposed to direct sunlight, allowing them to retain higher moisture levels (29).

In the present study, the active decay stage continued for five days in control corpses, while it showed a prolonged period of time and continued for 12 days in the atrazine-intoxicated group. Similarly, the active decay stage persisted for 4-6 days on rat carrions in Assiut, Egypt (27). While Abd El-bar et al. (16) reported 5 to 6 days for the active decay stage in the control and organophosphate-intoxicated rabbit cadavers, respectively. On the contrary, this stage was recorded to be 4 days in the control group and 3 days in the warfarin-intoxicated rabbit carcasses in a former report conducted in Cairo, Egypt (10).

The dry decay stage in the present study lasted for 7 and 14 days in the control, atrazine-intoxicated groups, respectively. Abd El-bar et al. (16) recorded that the dry decomposition stage continued for 12 to 30 days in the control and Pirimiphosmethyl-intoxicated rabbit cadavers, respectively in Egypt. Azmi et al. (30) reported the period of advanced decay stage as ten days on the rabbit carrions in Malaysia. This difference might be due to lack of exposure to the sun, besides the variation in carcass type. This was substantiated by Okiwelu et al. (31), who observed that the sun-exposed corpses decayed earlier than the shaded ones.

Notably, the decomposition in the atrazine-intoxicated carcasses was slower than in the control ones. This could be explained on the basis of delaying the beginning of insect succession and colonization in the atrazine-intoxicated cadavers that were observed in our study. In this context, the presence of drugs and toxins in a decomposing carcass has the potential to change the rate of insect invasion and immature stage development (16, 32). Our results were similar to those noticed for other pesticides. Abd El-bar et al. (9) reported that zinc phosphide intoxication delayed the decomposition of rabbit cadavers. Abd El-bar &Sawaby (16) observed a delay in rabbit carcass decomposition as a result of pirimiphosmethyl (organophosphate) poisoning. Sandoval et al. (33) noticed that the presence of pyrethroids and pyrethrins in rat corpses retarded decomposition. On the contrary, other studies reported that terbufos (organophosphate) and warfarin intoxication accelerated the decomposition process in rat and rabbit carcasses, respectively (10, 11). This acceleration might be owing to the fact that these

toxins did not conceal the corpse odor that is the major factor in attracting invading insects (16).

Insects and other arthropods are the main organisms implicated in the decomposition of the dead body. They arrive at the exposed remains immediately after death, usually in less than ten minutes, and rapidly start their activities (34). Our study revealed that several species of insects were detected on the control and atrazine-intoxicated carcasses. They were relatively few, with just two orders, eight families, and ten species represented in six species of Dipera belonging to four families, four species of Coleoptera belonging to three families. In a similar study, ten arthropod species and 7 families were detected on the rabbit and rat carcasses in Egypt (35). This observation may be attributable to the small size of the corpse, which could be quickly devoured by the early invader arthropods, depriving later colonizer species of the opportunity to colonize resulting in faster decomposition and shorter postmortem period (36). This finding was corroborated by Tomberlin et al. (37), who reported low insect diversity associated with small carrion size. In contrast, Aly et al. (38) recorded eighteen species of dipterous insects on rabbit cadavers in Egypt. A large animal carcass frequently attracts a large number of insect species, like the forty-seven species found in the report of Wang et al. (39). Abd El-bar et al (16) noticed 16 arthropod species on the rabbit carcasses intoxicated with pirimiphosmethyl (organophosphate) in El Qalyubiya Governorate, Egypt. This variation may be owing to different types of pesticides used.

The insect succession on the dead bodies is influenced by the stage of decomposition (40). Necrophagous insects are useful in forensic entomology for determining postmortem intervals, postmortem transmission, and the presence of toxins because they are always detected on decayed carcasses (10). In this study, Diptera were the first insect colonizers on the control carcasses. Blowflies have been observed to be attracted to corpses shortly after death; they can locate the corpse mostly by its odor (41). Nevertheless, the presence of atrazine delayed their arrival, and no insects were observed in any of the atrazine-intoxicated groups during the fresh stage. This observation was consistent with that of Jales et al. (11), who reported that Terbufos organophosphate retarded the insect succession and no insects were found on the intoxicated carcasses.

Calliphorids (blowflies) have been detected in all decomposition stages, excluding the fresh stage in the atrazine-intoxicated groups and the skeletal stage in all carcasses. Similarly, Abd El-bar et al (16) recorded a delay in the arrival time of Calliphoridae species in the organophosphate-intoxicated carrions, while Abd El-Gawad et al (10) observed Calliphorid flies on the warfarin-intoxicated cadavers during the fresh stage. These findings were in accordance with those of previous reports showing the presence of Calliphorids in all decomposition stages except the skeletal stage (17, 30, 42). On the other hand, some investigators noticed Calliphorid flies during the advanced decay and skeletal stages (29, 43), whereas Abouzied (36) found Calliphorids during the fresh and bloated stages only.

Chrysomya albiceps was the most common Calliphoridae species on all corpses. It was the key factor in the decay process, demonstrated a faster arrival, and is regarded as one of the most significant carrion feeding insects in various geographic areas, including Egypt (9, 17). This species is violent and can prey on other calliphorid larvae, which explains its predominance over other larvae of Calliphorids (44). *Chrysomya albiceps* arrived at the carcass within a few minutes after death, which could be important in estimating the PMI (45).

Sacrophagidae (flesh flies) were the second type of insect found on carrions after Chalhphodiae during the bloating stage in all studied groups, similar to a previous study (17). In contrast, El-Aziz et al (25) cited that Calliphoridae, Muscidae, and Sarcophagidae were found flying around the carcasses. Sarcophagidae and Calliphoridae have been described as the most common insect species that colonize dead bodies (46). Because of their prevalence and abundance on cadavers, they play an important role in forensic investigations (47). In this study, they invaded the corpses on the same Calliphorids colonization day, which was consistent with previous studies (9, 17). Sarcophagidae were recorded to have arrived and invaded the cadavers during the first two days of all of the various seasons (28). They

were collected from Terbufos-intoxicated rat carcasses in Brazil (11), and rabbit carrions intoxicated with pirimiphosmethyl (organophosphate) and zinc phosphide in Egypt (9, 16).

In our study, Sacrophagidae were less abundant than Calliphoridae during the day. This may be explained by the fact that Sarcophagids favor cooler periods and it has been recorded that they larviposit or oviposit at night only (39). The current investigation was carried out during the summer season, and the corpses were visited during the day, which explains the lower abundance of Sacrophagidae in this study. Fannidae were observed following Sarcophagids at the bloated stage in the control corpses only and during the active decay stage in the atrazine-intoxicated carcasses. Fannidae have been shown to be valuable in forensic investigations, particularly for determining the PMI (48).

Adult Piophilidae species are considered to be main pests in the food industry as well as myiasis agents (49). Piophilidae are frequently mentioned for their common existence in carrions (9). In the present study, the adult flies of piophilidae were detected first in the active decay stage on the atrazine-intoxicated corpses only, whereas their appearance was delayed until the dry decay stage in the control group. This may be owing to the stronger putrefaction odor in the atrazine-intoxicated group than in the control group.

Herein, Calliphoridae and Sarcophagidae were the most common dipterous maggots seen during the active decay stage. Dipterans lay their eggs on decayed fleshy tissue, and the eggs develop until the insects reach adulthood. Calliphorid Larvae have been found on the first day of the active decay stage in all groups, whereas Sarcophagidae larvae have been observed on the 2nd day in the control corpses. However, the larvae of *Wholfahrtia magnifica* and *Sarcophaga haemorrhoidalis* were seen on the atrazine-intoxicated cadavers on the 1st and 2nd days of active decay stage, respectively. Patterns of succession revealed that necrophagous blowflies maggots were active only in the active decay stage of decomposition. In contrast, Abd El-bar and Sawaby (16) demonstrated that these maggots were active in the 1st three decomposition phases, although not during the advanced decay stage. In addition, Martinez et al. (50) reported that Calliphoridae remained active on the pig corpses throughout the decomposition period (83 days).

In our study, the succession pattern of Coleopteran insects was different in the control and atrazine-intoxicated groups. They were first found during the bloated stage on the atrazine-intoxicated carcasses only (one adult species of Coleopteran Cleridae; *Necrobia rufipes*). The presence of coleopteran insects was remarkable during the active decay stage in both groups. The adult and larvae of coleopteran insects had been detected until the advanced decay and skeletal stages in the control and atrazine-intoxicated groups. These findings coincided with those of an earlier report (17). In this context, Abd El-bar et al. (16) observed that Coleopteran insect activity was completed throughout all phases of decomposition. This finding was substantiated by the findings of El-Aziz and El Shehaby (25), who noted that beetles are very adaptable and can be found in approximately all types of environments. Beetles favor the cadaver when it is more decayed or in a dry decay stage (17). In contrast, Abd El-bar et al. (9) found that cloepteran insects had been noticed at an active decay stage in the control and zinc phosphide-intoxicated rabbit carcasses. Coleopteran species are predatory insects that were observed in the current investigation during the existence of dipterous larvae, resembling the findings of Farag et al. (17) & Abd El-bar et al. (9). The presence of predatory beetles on corpses was correlated with the basic prey abundance. The activities of predatory beetles reach a climax during the active decay stage because the blowfly maggots are the most common prey in this stage of decomposition (51). The larvae and adults of dermestes beetles were found in later stages of decomposition when less flesh was still present. Dermestis are attracted to dried carcasses and they can feed directly on the carcass, contrary to rove beetles, which can speed up the decomposition process (52). We also recorded the presence of Cleridae *(Necrobia rufipes)* during the bloat stage and advanced decay stage of the atrazine-intoxicated carrions and control group, respectively. However, they had been noticed during the active decay stage in both groups. They are predators of some Piophilidae flies and Dermestidae larvae. Cleridae adults have been ob-

served to prey on Diptera larvae as well as carrions. Histeridae were also found in the active decay stage on control and atrazine-intoxicated carcasses, whereas they were detected during the skeletal stage in the control group only.

Of note, the presence of atrazine in intoxicated carcasses delayed the growth and development of insect larvae, consequently slowed down the decomposition process. This finding is corroborated with the observations of Yan-Wei et al. (53) who noticed that malathion retarded the development of flies from the larval to pupal stages. In this context, pyrethroid insecticides impair larval growth and development in bird cadavers (54).

Interestingly, we found that the Coleopteran insects (adults and larvae) dominate the proportion of dipterous insects (adults and maggots) in the atrazine-intoxicated groups by calculation of the proportion of both orders in the control and atrazine-intoxicated groups. This could be owing to the small number of dipterous larvae found on the intoxicated corpses, as well as the fact that Coleopteran insects are predators, besides some individuals that feed on the carcasses themselves. This observation was also recorded by Abd El-bar et al. (9) in zinc phosphide-intoxicated rabbit carcasses.

Conclusion

This work was successful in identifying several insect species of forensic importance on atrazine-intoxicated rat carcasses in Egypt during the summer season with respect to their succession pattern, diversity, and abundance. Therefore, in view of the importance of such insects, and their development on corpses for estimation of PMI as well as indication of suspicious poisoning, this study provides significant reports regarding the influence of atrazine on forensically important insects, which could be contributed to further investigations using other toxicants during different seasons.

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