ISSN 2347-6893
Volume 9 Number 1
Journalof Advances in Biology

SEASONALITY OF INSECT SUCCESSION ON REMAINS OF RABBITS TREATED WITH AMITRIPTYLINE (ANTIDEPRESSANT DRUG) IN ALEXANDRIA, EGYPT.

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ABSTRACT

In 2011 and 2012, seasonal field studies on vertebrate carrion decomposition and its associated arthropod fauna were carried out on sixteen healthy domestic rabbits, Oryctolagus cuniculus L., killed by asphyxia (control) and with different doses of a commonly used antidepressant drug amitriptyline (test). Amitriptyline involved in up to 33% of all fatal poisonings, second only to analgesics. Five decomposition stages were recognized: fresh, bloat, active decay, advanced decay and dry. Carcasses in summer and spring decayed at a much faster rate than in fall and winter. The presence of the amitriptyline in decomposing tissues, influences the development and pattern of carrion feeding insects and alter postmortem interval (PMI) estimates based on the rate of larval and puparial development. It should be pointed out that the presence of this drug alter the successional model in Alexandria, Egypt. Diptera was the predominant group, were as Coleoptera ranked 2nd. Calliphorids and Sarcophagids were the most important arthropod invaders of cacassess because of the influence they had on the rapid deterioration of the carcasses. It was of an interest to note that Formicidae was present in all carcasses, mostly in the early stages of decomposition. Carcasses which had been killed by different doses of amitriptyline were found to be unsuitable for certain flies. This study provide an additional knowledge in the context of Egyptian forensic entomology and the influence of amitriptyline which is of relevance to forensic science.

Indexing terms/Keywords

Forensic entomology – Postmortum interval – Arthropod succession – Antidepressant drug – Amitriptyline.

Academic Discipline And Sub-Disciplines

Entomology-forensic entomology.

INTRODUCTION

Forensic entomology is the science of collecting and analyzing insect evidence to aid in forensic investigations (Amendt et al., 2011). Medico-legal forensic entomology cover evidence that may be gathered through arthropod studies at events such as murder, suicide, rape, physical abuse and contraband trafficking (Catts and Goff, 1992). The typical questions posed to the medicocriminal entomologist involve estimating of the time a decedent has been dead (the "postmortem interval' or PMI) and less frequently, the place (situs) where death occurred (Hall, 2001). Together, arthropod succession and insect development have been successfully used to determine the PMI in criminal investigations (Amendt et al., 2004; Arnaldos et al., 2005; Matuszewski et al., 2010; Abd El-bar and Sawaby, 2011; Nazni et al., 2011).Succession of insects on carrion is forensically important for two reasons. Firstly, if the pattern of succession is known for a given set of circumstances, one may use it to estimate postmortem interval (PMI) with succession-based method (Goff and Flynn, 1991; Schoenly et al., 1992; Schoenly et al., 1996; Wells and LaMotte, 2001; Matuszewski et al., 2010). Secondly, knowledge on sequence and time of carcass colonization by immature of different taxa is necessary to properly place in time the minimum PMI estimated with development-based method (Smith, 1986; Benecke, 1998; Goff, 2000; Wells and LaMotte, 2001; Greenberg and Kunich, 2002; Arnados et al., 2005; Matuszewski et al., 2010). Succession studies need to be conducted in as many diverse geographic areas as possible, since there can be differences in insect fauna between geographic areas. In some cases, the insect fauna in a specific area can change over time. For example, the black soldier fly, Hermetia illucens (Linnaeus),was found for the first time in Italy as necrophagous in 1997 (Turchetto et al., 2001). Furthermore, insects may serve as important alternative species for toxicological analysis in cases where human samples are not available for this purpose (Goff and Lord, 1994; Verma and Reject Paul, 2013). Over the past several years, drug-related deaths have increased. It has been shown that the presence of substance such as drugs (cocaine, heroin, amitriptyline, etc.) and toxins in the carrion may serve to alter both the rate of development of individual insect species and the pattern of succession onto the carrion (El-Ghaffar et al. 2008). Tricyclic antidepressants (TCAs) are heterocyclic chemical compounds used primarily as antidepressants. The TCAs were first discovered in early 1950s and were subsequently introduced later in the decade (Carson, 2000). They are named after their chemical structure, which contain three rings of atoms (Figure 1). The TCAs include the following agents which are predominantly serotonin and/or norepinephrine reuptake inhibitor: Amitriptyline (Elavil, Tryptizol, Laroxyl). The severe morbidity and mortality associated with these drugs is well documented due to their cardiovascular and neurological toxicity. Additionally, it is a serious problem in the pediatric population due to their potential toxicity (Rosenbaum and Kou, 2005) and the availability of these in the home when prescribed for bed wetting and depression. Amitriptyline involved in up to 33% of all fatal poisonings, second only to analgesics (Thomas et al., 1996).

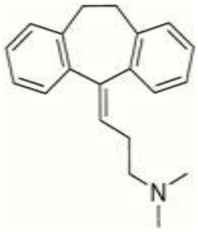

Figure 1: Chemical structure of the tricyclic antidepressant amitriptyline.

The objective was to identify the effect of amitriptyline on decompositional stages and arthropod succession patterns in different seasons. It is hoped that this work will help understanding the ecology of our local carrion arthropod community, and as a vital prerequisite to the application of medicocriminal forensic entomology in Egypt.

MATERIALS AND METHODS

In 2011 and 2012, seasonal field studies (summer, autumn, winter and spring) on vertebrate carrion decomposition and its associated arthropod fauna were carried out in botanical garden of Faculty of Science, Alexandria University, Egypt.Sixteen healthy domestic rabbits, Oryctolagus cuniculus L., weighing nearly (1.5 kg) were used in the study. Four rabbits were sacrificed for each season. Two methods were used in sacrificing the animals. The first method is asphyxiation by hanging to avoid external bleeding and to maintain their bodies intact (Tantawi et al., 1996; El-Ghaffar et al., 2008; Abd El-bar and Sawaby, 2011), the second method, the three received 12.8 mg/kg (0.5 LD50), 25.7 mg/kg (LD50), 51.4mg/kg (2 median lethal dose) of amitriptyline as antidepressant drug (Hughes and Radwan 1979).

Immediately after death, the carcasses were placed on the ground (Figure 2), so that the exact time of death was known. Placing and securing carcasses by an exclosure wire cage for easy lefting (Catts, 1992; El-Ghaffar et al., 2008) The four cages in each study were located ~1 meter apart. Four different areas were selected, to avoid faunal overlap among seasonal experiments. Sampling was conducted twice daily until it reach the dry stage, they were examined once daily. Weight of each carcass was recorded at each visit. Photographs of the carcasses in the various decomposition stages were taken. Observation was made of the physical condition and stage of decay of each carcass was noted. Areas of obvious insect activity were noted. Recorded of arrival, departure, and abundance of the attracted fauna were made for each of the species encountered. Collection and sampling were performed

An accurate identification of specimens is the first essential step in forensic studies. Morphological method are usually used (García-Rojo, 2004; Hwang and Turner, 2005; Baz et al., 2007; Matuszewski et al., 2010; Anton et al., 2011; Farinha et al., 2014). Almost all insect taxa were identified to the species level through the recognized keys available at the laboratories of the department of zoology, University of Alexandria. Climatological condition during the experimental periods is represented in. Daily ambient air temperatures and humidity were recorded from a maximum/minimum thermometer, set up in shaded place near the carcasses. Data on wind speed and rainfall were obtained from the meteorological station google weather (Table 1). Differences in succession patterns between carcasses were analyzed with respect to seasonal variations and environmental conditions, and concentration of used amitriptyline.

Conflict of interest:

The authors have declared no conflict of interest.

Compliance with ethics requirements:

All institutional and national guidelines for the care and use of animals (insects) were followed.

RESULTS AND DISCUSSION

Although the processes of decomposition and insect invasion are continuous, they are often described by discrete stages, which are characterized by the insect activity at each point in decomposition. The following decompositional stages described as followed: { Fresh stage (Figure 3), Bloated stage (Figure 4), Active decay stage (Figure 5), Advanced decay (Figure 6), Post-decay (dry) stage(Figure 7)}. There was a delay in decomposition process of tested carcasses relative to the control vary from 2 to 7 days according to season. This variation in the duration of this stage may be attributed to the differences in the daily mean temperature of the different season (Table 1). Similar variation was also observed by (Reed, 1958; Tantawi et al., 1996; El-Ghaffar et al.,2008) .

However, rare absence of bloating should be noted, as it indicates that putrefaction can decrease to such an extent that bloating will not occur. Previous studies have shown that bloating is influenced by ambient temperature and heat exchange parameters of the corpse (Korshunov et al., 2003). It is worth mentioning that, it was difficult to determine the end of dry stage due to its long duration and the lack of pronounced marking events, which was noted by various authors (Goff, 1992; El-Kady et al., 1994; Tantawi et al., 1996; Shalaby et al., 2000; El-Ghaffar et al., 2008; Abd El-bar and Sawaby, 2011). Indeed, the amitriptyline did not mask the odour of decomposition which attracted species to the carcasses, just as Voss et al. (2008) found for carbon-dioxide-poisoned carcasses. In contrast, El-Kady et al. (1994) found that neither decomposition nor arthropod invasion occurred on rabbits poisoned with arsenic oxide even after 11 months.

I-Order: Diptera:Twelve carrion breeders belonging to families: Calliphoridae, Sarcophagidae, Muscidae and Fanniidae, were found to breed on the rabbit carcasses and play the most important role in the process of carrion consumption, while Tantawi et al. (1996) noted 11 carrion breeding and El-Ghaffar et al. (2008) noted 14 carrion breeding Diptera on rabbit carcasses with the same pattern of arthropod succession observed in the present study. Figures 11-14 show the successional patterns of larval instars of these flies in different seasons. Smith (1986) noted that the presence of a particular adult fly species on the carrion does not necessarily mean that oviposition is occurring. The seasonal succession of the predominant flies breeding in rabbit carrion in the present study is recorded in Table 3. The term "primary", secondary" and "tertiary" were used in the present study to describe the sequence of flies infesting carcasses.

Family: Calliphoridae: Calliphora vicina (Robineau-Devoidy):C. vicina was absent during summer season . adult were only found in spring and autumn seasons, but breed only in winter season (Figures 12-14). The previous data indicate that this species prefers cooler temperature. Tantawi et al. (1996) reported that C. vicina was well represented in carrion in winter only ; indicated of a preference for cooler temperate.The duration of the immature stages of this species, on the control carcasses, was 22 days, while on treated carcasses, larvae first appear on day 15, post feeding larvae were first observed between day 26 and 28, puparia were noticed between day 28 and 39 (Figures 12). Therefore, the present data may indicate a longer minimum PMI estimate of 2 or 4-5 concerning larvae or pupae of this species, respectively. These prolongations were also noted by Goff et al. (1993), who showed an increased duration of the larval stages of Parasarcophaga ruficornis (Sarcophagidae) fed on tissues from rabbits receiving amitriptyline.

Lucilia sericata (Meigen): During the present study, L. sericata was a primarly fly in most seasons (Table 3) and was observed to breed on carrion in all season (Figures 11-14). These results agree with those of Ullyett (1950); Tantawi et al. (1996) and El-Ghaffar et al. (2008). In autumn (Figures from15 to 18), the duration of the immature stages of this species, on the control carcasses, was 22 days. Larvae were observed from day 8 till day 22. This is in agreement with Zumpt (1965), who stated that the feeding period of the larvae of L.sericata on a carcass was 5-11 days. L. sericata post-feeding larvae, in autumn, were observed on day 18, and last till day 20, when puparia were observed. This was also observed in laboratory studies by Kamal (1958) who found that the prepupal period of L.sericata lasted between 48 and 192 hours. Puparation was observed to last approximately 10 days, from day 20 till day 30. Kamal (1958) determined an average puparial period of 7 days (5-11days). Zumpt (1965) stated that the duration of pupal periods is prolonged with low temperature (Tantawi et al., 1996; El-Ghaffar et al., 2008).

The major observation during the present study was the prolongation of life spans of L. sericata in carcasses killed by different doses of the amitriptyline, in different seasons. The duration of the immature stages in spring 11 days, while in autumn and winter it was 22 and 24 days respectively, hence a minimum PMI estimate, using any developmental stage of L. sericata will be greater by 1-9 days depending on the stage of development. This observation agree with Goff et al. (1993).

Chrysomya albiceps (Wiedeman): In our study, the duration of Chr. albiceps from larvae to adult, in spring and summer seasons, was approximately 8-10 days. This was in agreement with Mackerras (1933) who noted that the period from egg to adult emergence is 12-14 days at 20°C.

In autumn season, was greatly prolonged, requiring almost 22 days. This is probably due to low temperatures, as this species prefers high temperatures (Greenberg and Povolny, 1971). (Figure 11,12,14). Chr. albiceps does not breed in winter, that in agreement with Ullyett (1950) who mention that in South Africa Chr. albiceps is a summer carrion breeder. Tantawi et al. (1996) found that, in Alexandria, Egypt, adults of this species occur only during the warmer months of the year.

Chrysomya megacephala (Fabricius): In the present study we observed Chr. megacephala on rabbit carcasses during warmer seasons only (spring and summer), the duration of the immature stages, on control carcasses, were 10 and 9 days, respectively. On the treated rabbit carcasses, Chr. megacephala observed breeding on carcasses killed with median and high lethal doses of amitriptyline, the duration of the immature stages was 12-13 and 12 days, during spring and summer, respectively (Figures 11,14). This may be due to the action of amitriptyline , which prolonged the larval development and consequently pupariation by 1-3 days , as noted by Goff et al. (1993).

Family: Sarcophagidae: Wohlfahrtia nuba (Wiedemann): In the present study, W. nuba invaded rabbit carcasses only in summer and autumn (Figures 11,12). Adults were entirely absent during winter and spring. This results in agreement with (Tantawi et al., 1996; El-Ghaffar et al., 2008). The duration of the immature stages on control carcasses were 8 days in summer. This species were only observed on rabbit carcasses which were treated with median lethal dose of amitriptyline with prolonged duration about 4 days (Figures 11, 13). This retardation may be attributed to the action of amitriptyline . This observation is in agreement with that of Goff et al. (1993) who stated that the larval duration of P. ruficornis was significantly longer when fed on tissues from rabbits receiving amitriptyline.

Sarcophaga aegyptiaca (Salem): It was observed in this study that S. aegyptiaca breeding on control rabbit carcasses only in autumn. The duration of the immature stage of S. aegyptiaca on the control carcasses, was 25 days (Figures 12). On the rabbit carcasses, treated with high dose of amitriptyline The duration of the post-feeding stage was retarded by 3 days and puparation period by 4 days.

Sarcophaga argyrostoma (Robineau-Desvoidy): S. argyrostoma was observed breeding on rabbit carcasses only in autumn and winter (Figures 12,13), during the present study. In autumn, the duration of the immature stages on the control carcass was 36 days. While carcasses of rabbit treated with different doses of amitriptyline, a prolongation of 5 days was recorded. This may be due to the action of amitriptyline. In winter season, S. argyrostoma were observed on control and treated carcass (Figures 13) the duration of the immature stages was 40 days on control carcass while it was 42 in treated rabbits.

Family: Muscidae: Synthesiomyia nudiseta (Wulp): In the present study, the adult hoovering on the control rabbit carcasses during all seasons, but were observed to breed only in autumn (Figures 12). The duration of the immature stages was 15 days. It should be noted that, it was difficult to observe any puparia under carcasses, since the puparia of this species are enclosed within a cocoon formed of seed grains cemented together (James, 1947 and Ferrar, 1980). These observations agree with (Tantawi et al., 1996; El-Ghaffar et al., 2008).

Muscina stabulans (Fallen): In the present study, M. stabulans was observed to breed only in winter (Figures 13). The duration of the immature stages, on the control carcass, was 22 days with a pupal period of 9 days. This agreement with Greenberg (1990) who stated that M.stabulans pupation period is relatively long (9 days).

Muscina prolapsa (Harris): M. prolapsa was observed only as larvae during winter season (Figures 13). This species was very rare as compared to M. stabulans. No feeding or post-feeding larvae were observed in later days for both carcasses.

Ophyra ignava (Harris): During the present study, adult of O. ignava were found on the rabbit carcasses in all seasons, but observed breeding only in autumn and winter seasons (Figures 12,13). this observation in agreement with Tantawi et al., 1996. The duration of the immature stages on the control carcass, In autumn (Figures 12), was 30 days but the pupae did not complete development to the adult stage. During winter season (Figures 13) the duration of the immature stages on control carcass was 35 days. However most of the larvae and post-feeding larvae were dead by the end of experiment, and no puparia of O. ignava were observed on treated carcasses.

Family Fanniidae: Fanniidae was presented in the present study by two species, Fannia leucostica (Meigen) and Fannia canicularis (Linnaeus), found only during winter and autumn seasons. Only the larvae of F. leucostica were observed in winter season. No postfeeding larvae or puparia of F. leucostica were observed on rabbit carcasses during winter season Adult of F. canicularis were observed in both seasons, but no larvae were observed on carcasses (Figures 13). Larvae of F. leucostica were observed on the control carcasses from day 23 to day 33 in winter. Similar observations were also noted for rabbits treated with amitriptyline (Figures 13).

II-Order Coleoptera: During the present study, 6 coleopetran species belong to five families were recorded in the field. They are: Staphylinidae, Histeridae, Cleridae, Tenebrionidae and Dermestidae were found in abundance and apparently played a regular part in the faunal succession on carcasses.

Family Staphylinidae: During this study, staphylinids visited rabbit carcasses in decay stages, were numerous dipterous maggot were present. In summer, the family was represented by Ph. Longicornis. In autumn, Staphylinidae were entirely absent. In winter, Staphylinidae were well represented by both species and was able to breed in carcasses. Payne and Crossley (1966) stated that immature staphylinids usually appeared too late to feed on maggots. In spring, Staphylinidae was only represented by adult Ph. Sordidus (Figures 11-14).

Family Histeridae: During the present study Histeridae frequented carcasses in the decay stages (active or/and advanced); in all seasons; and their feeding was confined to dipterouse larvae(Figures 11-14).They may be significant in reducing dipterous larvae in carrion (Nuorteva, 1970).

Family Cleridae: During the present study Cleridae frequented carcasses in the decay stages (active or/and advanced); in summer, winter and spring (Figures 11-14).

Family Tenebrionidae: During the present study, Tenebrionidae was represented by Blaps sulcata (Laporte). No larvae of this family were found on carrion and was only found in summer and autumn. In summer, B. sulcata was present on carrion during the bloat and decay (active and advanced) stages, while in autumn it was observed only during the bloating stage (Figures 11-14).

Family Dermestidae: Generally, dermestid beetles were present in carcasses from the bloat stage, active decay stage, advanced decay stage and through the dry stage. Dermestid beetles observed on carcasses in large numbers throughout the whole period of study and was able to breed in winter (Figures 11-14).

III-Order Hymenoptera: Family Formicidae: Pheidole megacephala (Fabricius) was the only ant species to frequent carrion during this study, where it acted primarily as an opportunistic member in the carrion community. It was the dominant arthropod species found on all carcasses. Ph.megacephala feeding actively during all stages of carrion decomposition. This ants was observed carrying off maggots and feeding on carrion itself. Similar observations of ants on carrion in other warmer temperate zones were noted by Reed (1958) and Payne (1965).

Family Vespidae: The oriental hornet, Vespa orientalis (Linnaeus), was the only Vespa species observed frequenting carcasses in the present study. This was visiting carrion during bloating stage and the early part of the decay stage in autumn only. Like ants vespa was an opportunistic species. It was observed feeding on the fluids seeping from carrion. Matsuura (1991) stated that insects are the main protein source for hornets.

IV-Other arthropod: During the present study, certain arthropod species belong to the order Embiidina, Lepidoptera, Araneae and Isopoda frequented rabbit carcasses. They appeared to play an unimportant role, except in the faunal succession on carrion and were attracted to carcasses incidentally or used the carcasses as an extension of their environment during their seasonal occurrence. In additional, none of these arthropods bred on carrion and most were few in number. However, certain groups such as silverfish and moths were characteristic fauna of the dry stage of decomposition in the cooler seasons (Figures 11-14).

Conclusions

Five decomposition stages were noticed at the rabbit carcasses killed by asphyxiation and different doses of amitriptyline. These stages are fresh, bloat, active decay, advanced decay and dry stages. It was found that, carcasses in summer and spring decompose at a much faster rate than those in autumn and winter. In summer, the carcasses took only from 7-9 days to reach the dry stage, while a period from 44-51 days was required to reach the same stage in winter. The prolongation was noticed as a result of injection of high doses of amitriptyline. The prolongation was especially noticed in cool seasons (winter and autumn) were it rang from 5 to 7 days, unlike in hot seasons (summer and spring) its only 2 days. The rare absence of bloating stage in autumn was noted, due to slow putrefaction process.

Three common major temporal events were noticed among the seasonal arthropod succession patterns in this study. The first of which included rapid invasion of carcasses by adult dipteral (mainly calliphorids and sarcophagids) and ants. The second event was characterized by the presence of dipterous larvae and adult Coleoptera, during which arthropod diversity reached its maximum. A distanced decline in arthropod richness marked the third event, when most species migrated away from the carcasses. A difference in carrion-arthropod communities was observed as a result of amitriptyline doses used at a specific seasons. It is of interesting to indicate that rabbit carcasses, which had been killed by different doses of amitriptyline were found to be unsuitable for certain flies to breed such as Synthesiomyia nudiseta "Muscidae".

The manner of death had induced several noticeable differences in the duration of the development of the immature stages of Calliphoridae, Sarcophagidae, Muscidae and Fanniidae. It may be concluded that the presence of the drug (amitriptyline) in decomposing tissues, influences the development and pattern of carrion feeding insects and could alter PMI estimates based on the rate of larval and puparial development. It is worth mentioning that Calliphorids and Sarcophagids were the most important arthropod invaders of carrion because of the influence they had on the rapid deterioration of the carcasses. They developed rapidly, colonizing and consuming the carcasses soft-tissues, besides being attractive preys for other predatory arthropods.

Staphylinids, Histerids and Tenebrionids were the main coleopteran predators of dipterous larvae. Members of the two former families arrived at carrion during the active decay stage of decomposition, while Blaps sulcata was only active at night during the bloated stage in summer and autumn. The dermestid beetles frequented carrion during the whole period of study. It arrived early during the bloated stage of decomposition. The ant species Pheidole megacephala (Fabricius) was the main opportunistic arthropod during all stages of carrion decomposition; on both control and treated carcasses in all seasons. Apparently, it is clear that the death by amitriptyline had no effect on the visiting and the feeding pattern of Hymenoptera, Embiidina, Lepidoptera, Araneae and Isopoda. The absence of some species during any experiment was mainly due to their opportunistic feeding habit rather than the effect of amitriptyline.

The lower number of species attributed to fact that Egypt in general has a limited species diversity; and studing area (botanical garden) is a fragmented habitat not a continuation of a major habitat, which further limits diversity. There are 27 arthropod species belong to 7 orders (Diptera, Coleoptera, Hymenoptera, Embiidina, Lepidoptera, Araneae and Isopoda) and 16 families were recorded on rabbit carrion. However, some species were incidental visitor, and not involved in the decomposition process such as Araneae.

Table 1: Climatological data recorded during the experimental period.

Season	Date	Temperature (°C)			Relative Humidity %	Total rain fall
		Max.	Mini	Mean		
Summer	1-15 August 2011	$36^{\pm 2.5}$	$21.2^{\pm 1}$	$28.6^{\pm 1.75}$	77.1	00
Autumn	1-30 November 2011	$19^{\pm 1.2}$	$10.5^{\pm 1.4}$	$14.75^{\pm 1.3}$	63.8	22.4
	1-30 December 2011	$17^{\pm 1.6}$	$9.2^{\pm 0.5}$	$13.1^{\pm 1.05}$	65.3	20.1
Winter	1-30 January 2012	$17^{\pm 1.1}$	$8.5^{\pm 0.9}$	$12.75^{\pm 1}$	70.8	18.9
	1-29 February 2012	$18^{\pm 1.3}$	$9^{\pm 1}$	$13.5^{\pm 1.15}$	69.2	12.1
	1-20 march 2012	$18^{\pm 1.1}$	$10^{\pm 1.2}$	$14^{\pm 1.15}$	67.7	4.1
Spring	1-22 may 2012	$20.1^{\pm 1.4}$	$29.5^{\pm 1.5}$	$24.8^{\pm 1.45}$	60.2	00

Table 2:Seasonal variation in the duration of each decomposition stage of rabbit carrions treated by amitriptyline in the experiment (By days).

Seasons	Stages of decomposition	Control	1/2 LD_{50}	LD_{50}	2 LD_{50}
Summer	Fresh	0-1	0-1	0-1	0-1
	Bloat	1-2	1-3	1-3	1-3.5
	Active decay	2-4	3-5.5	3-5.5	3.5-6
	Advanced decay	4-6	5.5-8	5.5-8	6-8
	Dry*	D_7	D_9	D_9	D_9
Autumn	Fresh	0-3	0-3	0-3	0-3
	Bloat	3-9	3-9	3-10	escaped
	Active decay	9-16	9-17	10-18	3-20
	Advanced decay	16-25	17-26	18-27	20-30
	Dry*	D_{26}	D_{27}	D_{28}	D_{31}
Winter	Fresh	0-6	0-6	0-6	0-7
	Bloat	6-16	6-16	6-17	7-18
	Active decay	16-28	16-30	17-32	18-34
	Advanced decay	28-43	30-45	32-48	34-50
	Dry*	D_{44}	D_{46}	D_{49}	D_{51}
Spring	Fresh	0-2	0-2	0-2	0-2
	Bloat	2-5	2-5	2-6	2-6
	Active decay	5-7	5-7	6-9	6-9
	Advanced decay	7-10	7-10	9-12	9-13
	Dry*	D_{11}	D_{11}	D_{13}	D_{14}

* Due to difficulty in determining the end of this stages, only the initial day is recorded

Table 3:Seasonal succession of the dominant flies breed in rabbits carrion in Alexandria, Egypt.

	Sequence	Summer	Autumn	Winter	Spring
Control rabbit	Primary flies	*Wolfahrtia nuba*	*Sarcophaga argyrostoma* *Sarcophaga aegyptiaca* *Lucilia sericata* *Wohlfahrtia nuba*	*Calliphora vicinia* *Lucilia sericata*	*Lucilia sericata*
	Secondary flies	*Chrysomya albiceps*	*Chrysomya albiceps* *Synthesiomyia nudiseta*	*Muscina stabulans* *Muscina prolapsa*	*Chrysomya albiceps*
	Tertialy flies		*Ophyra ignava*	*Ophyra ignava* *Fannia leucosticta*	
1/2 LD_{50} rabbit	Primary flies	*Chrysomya albiceps*	*Lucilia sericata*	*Lucilia sericata*	*Lucilia sericata*
	Secondary flies		*Chrysomya albiceps*	*Muscina stabulans*	*Chrysomya albiceps*
	Tertialy flies		*Ophyra ignava*	*Fannia leucosticta*	
LD_{50} rabbit	Primary flies	*Wolfahrtia nuba*	*Lucilia sericata*	*Lucilia sericata*	*Lucilia sericata*
	Secondary flies	*Chrysomya albiceps*	*Chrysomya albiceps*	*Muscina stabulans*	*Chrysomya albiceps*
	Tertialy flies		*Ophyra ignava*	*Fannia leucosticta*	
$2LD_{50}$ rabbit	Primary flies	*Chrysomya albiceps*	*Lucilia sericata*	*Lucilia sericata*	*Lucilia sericata*
	Secondary flies		*Chrysomya albiceps*	*Muscina stabulans*	*Chrysomya albiceps*
	Tertialy flies		*Ophyra ignava*	*Fannia leucosticta*	

Figure 2: The study area and placement of carcasses.

Figure 3: Fresh stage.

Figure 4: Bloated stage.

Figure 5: Active decay stage

Figure 6: Advanced decay stage.

Figure 7: Dry stage

(a) (a) In cold season

(b) In worm season

Figure 8: Comparison between bloat stage in:

(a) the warm seasons

(b) the cool seasons.

Figure 9. Rabbit carcass showing that upper abdomen decay faster than lower abdomen.

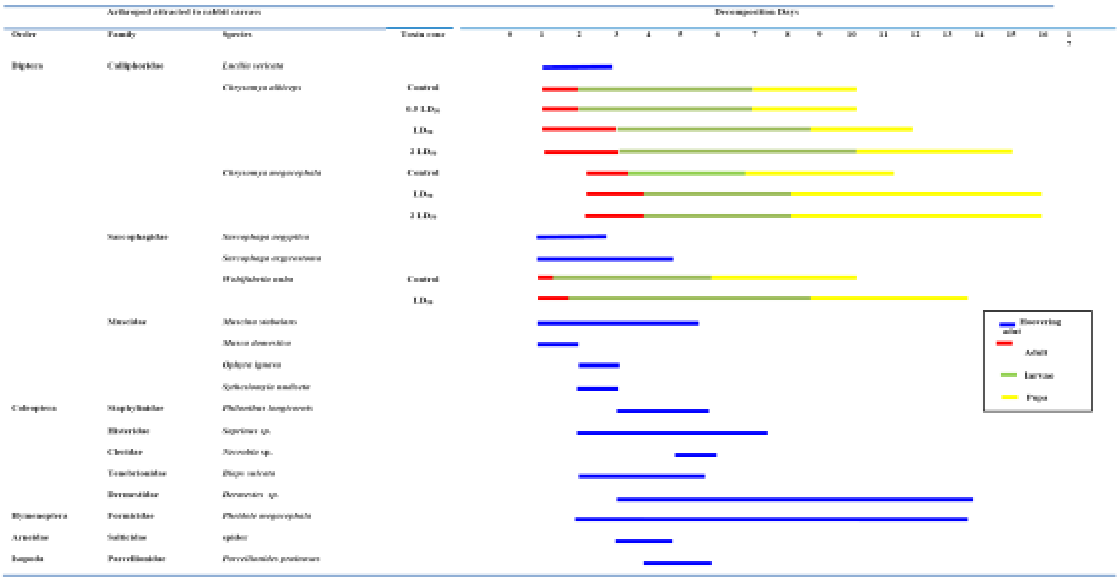

Figure (11): Arthropod succession on exposed rabbit carcasses during summer in Alexandria, Egypt. (1-15 August 2011).

ISSN 2347-6893
Volume 9 Number 1
Journalof Advances in Biology

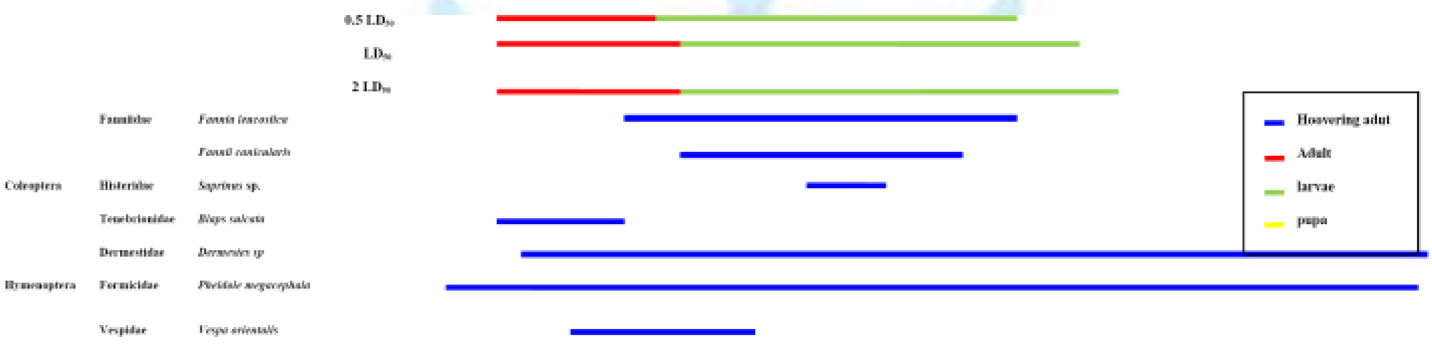

Figure (12): Arthropod succession on exposed rabbit carcasses during autumn in Alexandria, Egypt. (1 November - 30 December 2011).

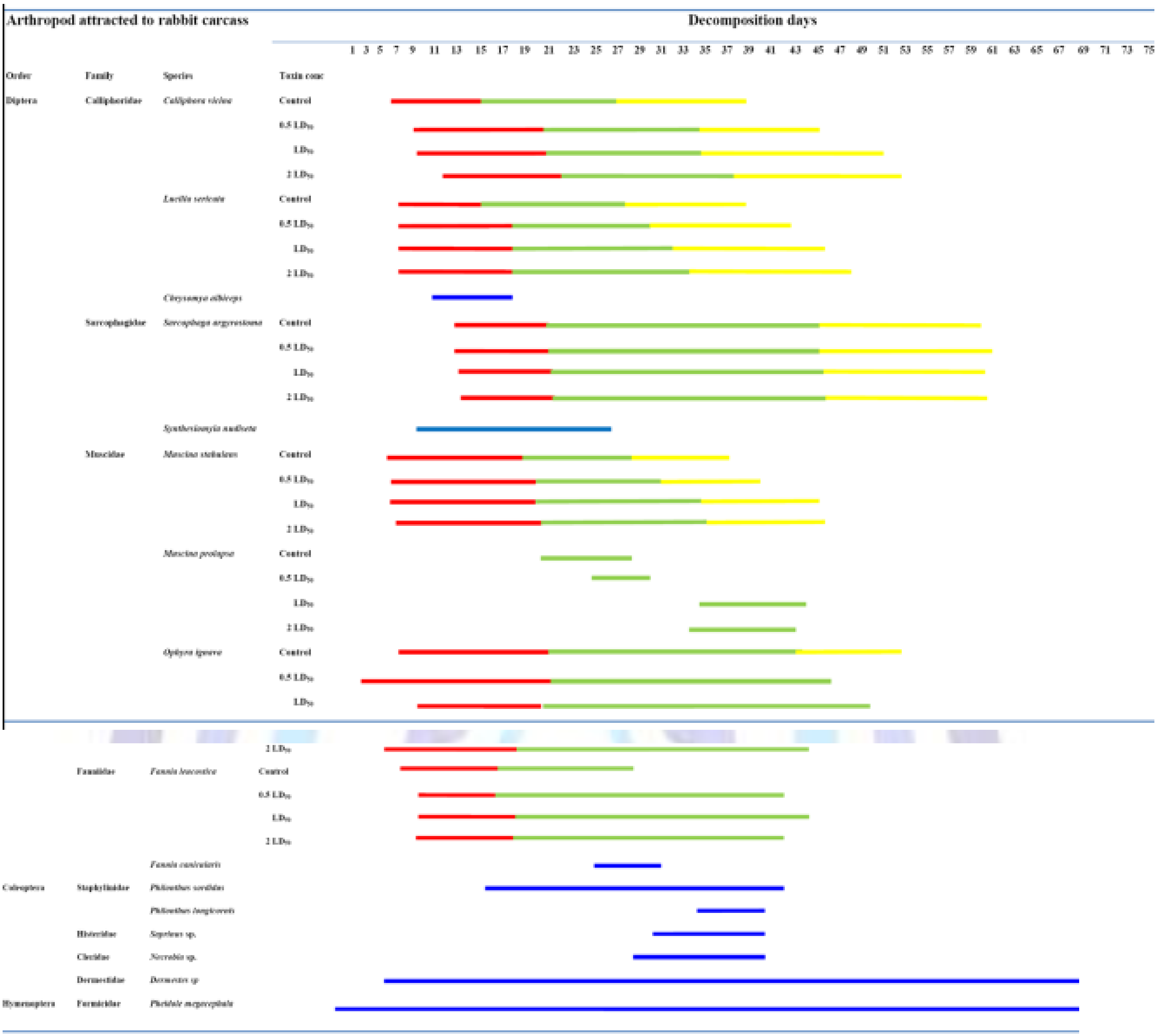

Figure (13): Arthropod succession on exposed rabbit carcasses during winter in Alexandria, Egypt. (1 January-20 March 2011).

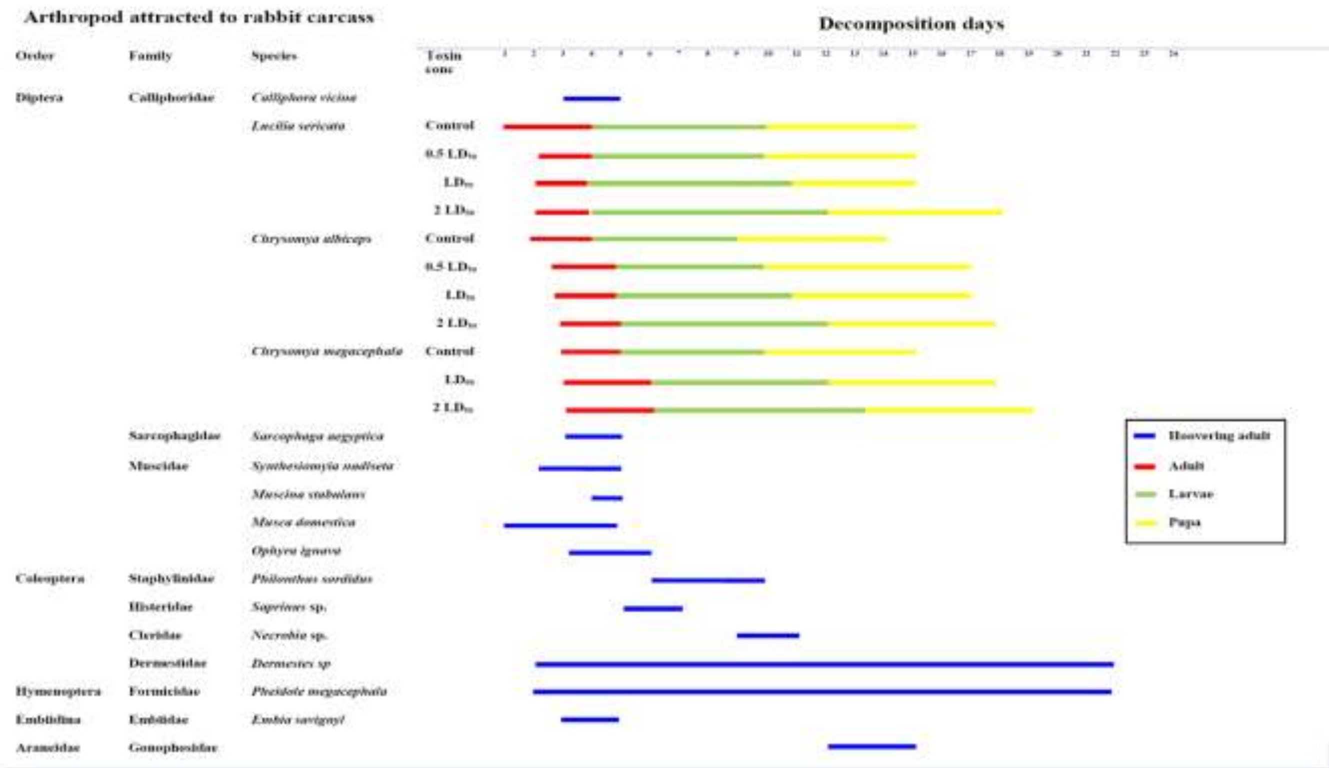

Figure (14): Arthropod succession on exposed rabbit carcasses during spring in Alexandria, Egypt. (1-22 May 2012).

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