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DIVERSITY AND SUCCESSION PATTERN OF FORENSICALLY IMPORTANT DIPTERAN SPECIES ASSOCIATED WITH ORGANOPHOSPHATE PESTICIDES-INTOXICATED RAT CARCASSES IN SARAWAK, MALAYSIA

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ABSTRACT

This research aimed to study the decomposition process of organophosphate-intoxicated rat carcasses and the succession pattern of associated insects at a secondary forest in Kuching, Sarawak, Malaysia. The animal model, *Rattus norvegicus,* weighing around 180-200g were assigned in T1 and T2 groups and were given LD_{50} dosage of organophosphate, glyphosate, and chlorpyrifos via oral administration, respectively. Sixty minutes after oral administration, the rats were euthanized and brought to the study site. The rat carcasses were monitored daily and the carrion insects were collected throughout the decomposition process. From five trials of experiments, a total of 1454 individual flies were collected, belonging to three families and 10 species. The highest number of fly species collected was *Chrysomya rufifacies* (75.03%) being the predominant species infesting the carcasses. The dipteran diversity and succession pattern were similar for all groups of carcasses, even though insect abundance were the least in T2 carcasses. Five stages of decomposition were observed in all rat carcasses, with longer decomposition duration in intoxicated carcasses (T1: 7.85±0.51 and T2: 15.8±2.82 days) compared to the control group (7.25±0.59 days). In conclusion, the organophosphate has altered the decomposition duration and the number of flies infesting the carcasses especially on chlorpyrifos-intoxicated carcasses. This work provides relevant information regarding the insect's succession pattern and the changes in the decomposition period which may assist in the determination of post-mortem interval time in future investigation processes when organophosphate poisoning is suspected.

Keywords: Forensic entomology, organophosphate, decomposition, insect succession pattern

ABSTRAK

Penyelidikan ini bertujuan untuk mengkaji proses penguraian bangkai tikus yang diracun dengan organofosfat dan corak sesaran serangga yang berkaitan di hutan sekunder di Kuching, Sarawak, Malaysia. Model haiwan, *Rattus norvegicus* dengan berat sekitar 180-200g

dibahagikan ke dalam kumpulan T1 dan T2 dan diberi dos LD_{50} organofosfat, glifosat, dan klorpirifos masing-masing melalui pemberian oral. Setelah 60 minit pemberian secara oral, tikus tersebut dimatikan secara eutanasia dan dibawa ke lokasi kajian. Bangkai tikus dipantau setiap hari dan serangga pemakan bangkai dikumpulkan sepanjang proses penguraian. Dari lima percubaan eksperimen, sejumlah 1454 individu lalat dikumpulkan, yang terdiri daripada tiga famili dan 10 spesies. Jumlah spesies lalat tertinggi yang dikumpulkan adalah *Chrysomya rufifacies* (75.03%), iaitu spesies utama yang memakan bangkai. Kepelbagaian Diptera dan corak keberjayaan serangga adalah serupa untuk semua kumpulan bangkai, walaupun jumlah serangga paling sedikit di bangkai T2. Lima peringkat penguraian diperhatikan pada semua bangkai tikus, dengan jangka masa penguraian yang lebih lama dalam bangkai yang diracun (T1: 7.85±0.51 dan T2: 15.8±2.82 hari) berbanding dengan kumpulan kawalan (7.25±0.59 hari). Kesimpulannya, organofosfat telah mengubah jangka masa penguraian dan jumlah lalat pemakan bangkai terutama pada bangkai yang diracun oleh klorpirifos. Hasil dapatan kajian ini memberikan maklumat yang relevan mengenai corak sesaran serangga dan perubahan dalam tempoh penguraian yang dapat membantu dalam penentuan selang masa kematian dalam proses penyiasatan pada masa hadapan ketika keracunan organofosfat disyaki.

Kata kunci: Entomologi forensik, organofosfat, penguraian, corak keberjayaan serangga

INTRODUCTION

In forensic entomology, several factors were taken into account in estimating the $_{min}$PMI which include the decomposition stages, insect succession pattern, and insect developmental stages (Catts & Goff 1992; Oliveira -Costa & Mello-Patiu 2004). These factors are closely related because the decomposition process is heavily influenced by the organisms that feed on the body. During each stage of decomposition, the varying in biological, chemical, and physical changes that occur throughout the decay process will attract different species of insects, for example scuttle flies (Zuha & Disney 2023), blowflies, flesh flies and carrion beetles (Anderson & VanLaerhoven 1996).

However, the presence of drugs or poisons in the body can affect the estimation of $_{min}$PMI (Goff & Lord 1994). Several studies investigated the effects of poisonous substances such as organophosphate (Abd El-bar & Sawaby 2011; Jales et al. 2020), ethanol (Tabor et al. 2005), gasoline (Rumiza et al. 2010), and morphine (Bourel et al. 1999). From their studies, the chemical or toxic substances not only affected the insects' arrival time and colonisation time, but it was also found that the chemicals interfered with the development of the insects that were infesting on the body (Carvalho et al. 2001; Mahat et al. 2009; Wolff et al. 2004). Pesticide poisoning is not new in Malaysia. A study conducted by the National Poison Centre of Malaysia from 2006 to 2015 showed an increasing trend in pesticide poisoning incidents over the 10-year duration with the highest number of poisonings being due to herbicides (44%) followed by agriculture insecticides (34%). Among herbicides poisoning, 53% are caused by glyphosate and chlorpyrifos was the top agent involved in poisoning among organophosphate insecticides (Kamaruzaman et al. 2020). This may be caused by the availability of the pesticides as it used for agricultural purposes which makes them one of the main causes of poisoning by accidental exposure, suicides and sometimes homicide (Sungur & Guven 2001).

This present study aims to observe the effect of organophosphate pesticides on the decomposition process of carcasses and to establish the baseline data for fauna succession populating decomposing rat carcasses in a secondary forest in Kuching, Sarawak, Malaysian Borneo.

MATERIALS AND METHODS

Study Area

The study was conducted in a secondary forest at Kampung Sri Kandong, Jalan Taman Negara Kubah, Kuching, Sarawak, Malaysia (1°38'05"N, 110°09'34"E, 24 m) (Figure 1) from November 2020 until November 2021. The one-acre land is located approximately 30 km north west of Kuching city. The land is a hill forest land with red-yellow sand and clays soils. The hill located close to sandstone mountains which are Gunung Selang and Gunung Serapi of Matang range. The habitat is a secondary forest surrounded by smaller trees and dense ground vegetation.

Figure 1. The figure showing a zoom-in map of Kuching, Sarawak. The location of the study site for the experiment was labeled accordingly

(Source: Adapted from Google Map 2023)

Based on the environmental data provided by Meteorological Department of Malaysia (MET Malaysia), the annual ambient temperature in Kuching was ranged from 23 to 33°C (annual mean: 27°C). Kuching receives a lot of rain even in the driest month, where the average rainfall accumulated at 2435.1 mm yearly. The weather station is located at Kuching Airport, which was 35 km southeast from the study site. In this study, the ambient temperatures and relative humidity was recorded *in situ* using data logger (Extech RHT20, Malaysia).

Pesticides and Animal Preparation

Two types of pesticides were used in this study which is, herbicide: glyphosate (30.5%) (Asset®, registration number: LRMP.R1/3106) and insecticide: chlorpyrifos (38.7%) (KENSBAN 400®, registration number: LRMP.R1/4644). The pesticides for each category were commonly used in agriculture and can be purchased at a local agriculture shop. Twelve male albino rats (*Rattus norvegicus* Berkenhout, 1769 var. WISTAR), three to four months old

were used as the animal model. Each rats weighing around 180-200 g. The rats were purchased from a local rodent farm in Kuching, Sarawak and was healthy upon delivery. The rats were kept in cages in laboratory for at least 72 hours for acclimation prior to experiment (Conour et al. 2006).

The experimental design for this study followed the procedure by Dupont et al. (2011) with modification. The rats were divided into three groups which consisted of control (C), glyphosate-intoxicated (T1) and chlorpyrifos-intoxicated (T2) rats (Table 1). All the rats were intoxicated with pesticides (LD_{50} dosage) according to designated groups, respectively, through oral administration procedure following the animal ethics guideline. The pesticides were allowed to be absorbed in the rats' body for 60 minutes before the rats were euthanized by cervical dislocation. The pesticide administration procedure was performed by a trained medical laboratory technologist of the Faculty of Medicine and Health Sciences. This research has obtained animal ethics approval from the Animal Ethic Committee UNIMAS (UNIMAS/AEC/F07/051).

Table 1. Description of each group of rats in each trial of experiment

Group	**Description**	**Treatments**
Control	Negative control	Distilled water
T1	Treatment 1	Glyphosate
T2	Treatment 2	Chlorpyrifos

Experimental Design

Rat carcasses were brought to the study site immediately which took about 40 minutes. The rats were wrapped in double plastic bags and kept in cooler box during transportation. At the site, the rats that were in the same group was placed at least one meter from each other, and >20 meters in between groups (Figure 2). The carcasses were covered with slotted plastic baskets to avoid disturbance by other predators and at the same time allow the insect access towards the carcasses. The carcasses were visited every day at the same time in the morning (~11 a.m), continuously every day until the carcasses were completely decomposed (1-3 weeks). During each visit, the physical changes of the carcasses during the decomposition process, and flies visiting and infesting the carcasses were observed and noted. The adult and immature flies were collected for species identification.

The experiment was repeated. Each trial (replication) took around a month with at least two weeks interval between trials. The site of experiment was conducted at the same location in each trial.

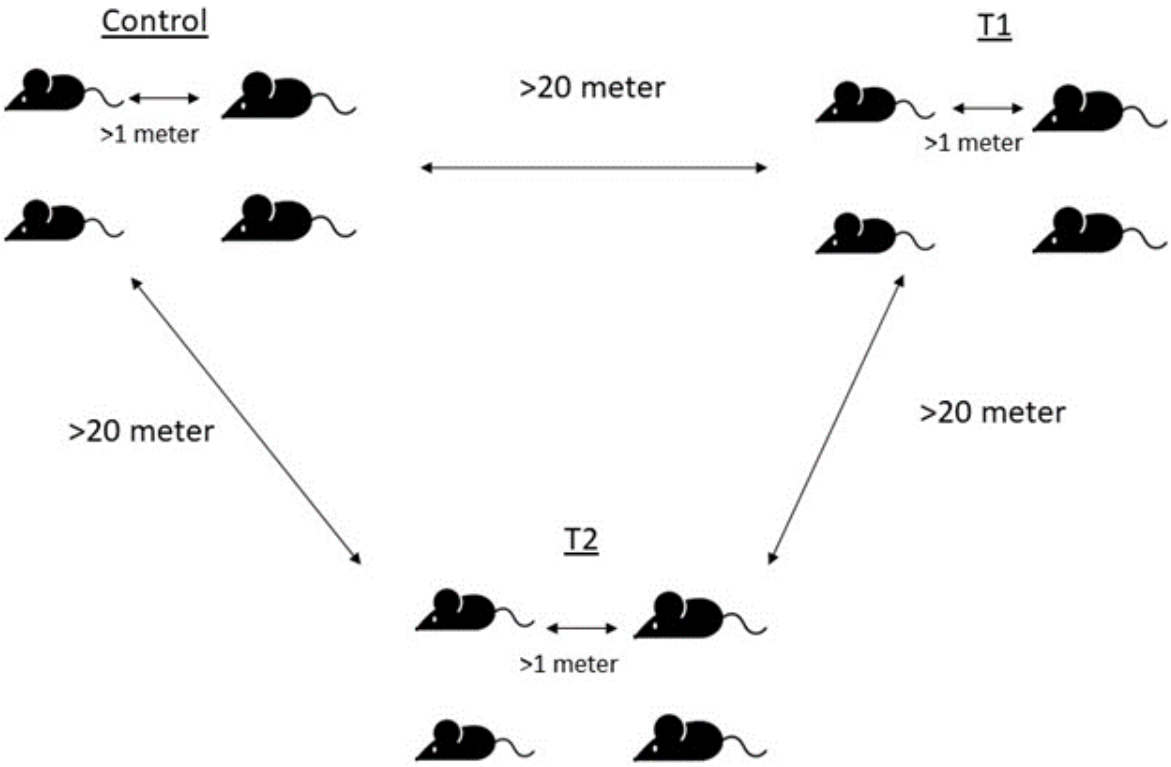

Figure 2. The experimental design for field experiment consisted of four rats in each group. Each rat was arranged one meter apart from each other, and >20 meter apart between groups. The experimental design follows method by Dupont et al. (2011) with modification

The Adult Flies Collection and Identification

Flies associated with the carcasses were monitored and collected for preservation and identification. The adult flies were caught using a sweep net, while crawling fly larvae were sampled by hand. The adult insects were then killed by putting them in a killing jar. The killing jar consisted of a round glass jar with a screwcap, containing cotton balls soaked with ethyl acetate. Ethyl acetate is used because it can retain the morphology of the flies. Then, the killed adult flies were pin using an insect pin (BioQuip, USA) and examined under a stereomicroscope Olympus SZ2-ILTS equipped with an Olympus DP27 camera. The flies' species identification was based on the description and key identification by Clearwater (1981) and Nazni et al. (2011).

Fly Larvae Collection and Identification

Fly larvae infesting on carcasses were caught using hands and put into a labelled specimen container. Twenty of the larvae collected were killed in hot water (80°C) and kept in 70% ethanol for further mounting process for species identification. The procedure followed the method by Heo et al. (2015).

Meanwhile, another 20-30 larvae collected were reared into adults to confirm the mounted larvae species identification. The larvae were put into a 1L beaker containing wood shaven and fed with minced meat. Once the larvae are in the prepuparial stage, they crawled

into sawdust to pupate. The pupae were monitored and the emerged adults were killed and identified for their species.

Carrion Decomposition and Environmental Parameter Analysis
The physical changes in carcasses throughout the decomposition process on the field were photographed during each visit using a smartphone camera (Huawei Mate20 Pro). The decomposition stages classification was determined based on criteria described by Goff (2009). The daily ambient temperature and relative humidity data at the study site were recorded using data logger (Extech RHT20, Malaysia).

Data Analysis
The comparison of environmental data between trials was analysed using One-way ANOVA (SPSS ver. 27). The entomological data was tabulated in a table using Microsoft Office 2016 and analysed using diversity indices below.

Relative abundance of species (%)

$$\text{Relative abundance} = \frac{\text{The abundance of one species (ni)}}{\text{Total all species counted (N)}} \text{ x } 100$$

Shannon Weiner's index

$$H = -\Sigma\,(pi \ln pi)$$

where,
pi = is the proportion of individuals found in species i.
ln = is the log with base 'e'

Shannon effective number of species

$$ENS = \exp[-\Sigma\,(pi \ln pi)]$$

Meanwhile, comparison of carcasses decomposition period between groups across five trial of experiments was analysed using multivariate analysis of variance (MANOVA) with significant level of p= 0.05.

RESULTS

Environmental Parameters at the Study Site
The study was conducted in five trials of experiments from November 2020 until November 2021. Throughout the study period, the mean ambient temperatures ranged from 27.6±0.6 to 29.5 ±0.5°C and the relative humidity ranged from 69.7±3.7 to 76.5±3.8%. Statistical analysis (one-way ANOVA) had shown no significant difference of ambient temperatures [$F_{4,80}$ = 1.889, *P = 0.120*] and humidity [$F_{4,80}$ = 0.698, *P = 0.595*] in between trials (Table 2).

Table 2. The environmental data throughout the study period from November, 2020 – November, 2021. The data was provided by Malaysia Meteorological Department (MET)

Trials	Intervals	Daily Surface Temperatures (°C) (Mean±S.D.)	Average Relative Humidity (%)	Accumulated Rain (mm)
Trial 1	23rd Nov -17th Dec	28.4±0.6	76.1±2.8	129.1
Trial 2	21st Jan -15th Feb	27.6±0.6	69.7±3.7	322.0
Trial 3	3rd -20th March	28.5±0.4	76.5±3.8	181.7
Trial 4	22nd Sept -11th Oct	29.5±0.5	73.8±2.4	105.0
Trial 5	10th -30th Nov	28.0±0.6	72.3±4.0	103.1

Dipteran Diversity

A total of 1454 individual dipteran flies which belongs to three families and ten species were collected from all groups of rat carcasses over the study period. There was a total of 670, 604 and 180 insects collected in control group, T1 and T2, respectively (Table 3). Statistical analysis had shown that there was a significant difference in insects population between groups of carcasses [$F_{2,12} = 5.136$, $P = 0.024$]. Significantly lower insects were observed in chlorpyrifos treated group ($P<0.05$) in five trials of experiments.

Among 10 species of flies collected, control group showed the highest species richness with 10 species of insects collected, followed by 7 and 6 of insect species in glyphosate and chlorpyrifos treated carcasses respectively (Table 3). Calliphoridae was the highest insects collected on carcasses which accounted for 92.78% of all flies collected in which dominated by *Chrysomya rufifacies* (Macquart 1843) (75.03%). followed by *Chrysomya megacephala* (Fabricius 1794) (12.79%) and *Hemipyrellia ligurriens* (Wiedemann 1830) (4.68%). These species were among the early insect species to arrive. Other calliphorids; *Lucilia* sp., *Lucilia cuprina* (Wiedemann 1830) and *Hypopygiopsis violacea* (Macquart 1835) were present in small numbers (<1%) in control carcasses and absent in all pesticide-intoxicated carcasses. Besides calliphorids, other families such as Sarcophagidae and Muscidae flies were also recovered from rat carcasses which accounted for 1.86% and 5.36%, respectively.

Hydrotaea chalcogaster (Wiedemann 1824) was the most common muscid fly found on the carcasses in all groups (Total=45), followed by *Atherigona orientalis* (Schiner) (Total=19) and *Musca domestica* (Linnaeus) (Total=14) which were collected during late decomposition stages.

There is no significant difference in the diversity of insect species between groups ($F_{2,12} = 1.401$, $P = 0.284$). However, insects collected on chlorpyrifos-treated carcasses shows the highest diversity index (1.304), although it had the least abundance insect species. The Shannon effective number of species (ENS) of chlorpyrifos (3.685) and the control group (2.123) was higher compared to insect species in glyphosate treated groups (1.632). This indicate the insect species visiting carcasses both in control and chlorpyrifos treated group was more diverse.

Table 3. Relative abundance and species diversity of dipteran collected in control and organophosphate pesticide-intoxicated carcasses groups

Order	Family	Genus/Species	Control		*T1		*T2		Total	RA
			N*	RA*	N	RA	N	RA		
Diptera	Calliphoridae	*Chrysomya rufifacies*	541	80.75	488	80.79	62	34.44	1091	75.03
		Chrysomya megacephala	52	7.76	53	8.77	81	45.00	186	12.79
		Hemipyrellia ligurriens	48	7.16	20	3.31	0	0.00	68	4.68
		Lucilia cuprina	1	0.15	0	0.00	0	0.00	1	0.07
		Lucilia sp.	2	0.30	0	0.00	0	0.00	2	0.14
		Hypopygiopsis violacea	1	0.15	0	0.00	0	0.00	1	0.07
	Sarcophagidae	*Sarcophaga sp.*	1	0.15	23	3.81	3	1.67	27	1.86
	Muscidae	*Hydrotea chalcogaster*	16	2.39	12	1.99	17	9.44	45	3.09
		Musca domestica	1	0.15	3	0.50	10	5.56	14	0.96
		Atherigona orientalis	7	1.04	5	0.83	7	3.89	19	1.31
Total		10	670	100.00	604	100.00	180	100.00	1454	100.00
Diversity index (H)			0.753		0.767		1.304			
Shannon ENS			2.123		1.632		3.685			

*N= number of individual species
*RA= Relative abundance
*T1= Glyphosate-intoxicated carcasses group
*T2= Chlorpyrifos-intoxicated carcasses group

Carcass Decomposition and Dipteran Succession Pattern

Five decomposition stages were observed in all groups of carcasses, which are fresh, bloated, active decay, advanced decay, and dry remains. The duration of decomposition for pesticide-intoxicated rat carcasses was relatively longer as compared to the duration of decomposition for the control group. The control group recorded duration of decomposition of 7.25±0.59 (Mean±SD) days to complete while the decomposition of rat carcasses treated with glyphosate (T1) and chlorpyrifos (T2) recorded 7.85±0.51 days and 15.8±2.82 to complete, respectively (Table 4).

Table 4. The duration of rat carcasses decomposition treated with glyphosate (Group T1) and chlorpyrifos (Group T2)

Stages of decomposition	**Days (Mean±SEM)**		
	Control	**Group T1**	**Group T2**
Fresh	1.00±0.00	1.20±0.41	1.30±0.47
Bloated	1.40±0.50	1.60±0.50	2.65±0.81
Active decay	2.40±0.60	2.30±1.03	8.00±1.71
Advanced decay	1.45±0.51	1.75±0.79	2.85±0.93
Dry remain	1.00 0.00	1.00±0.00	1.00±0.00
Total	**7.25±0.59**	**7.85±0.51**	**15.8±2.82**

The fresh stage started after the rats were euthanized and lasted for about 24 hours. The earliest visitors on rat carcasses in all groups were blowflies; *Ch. rufifacies Ch. megacephala, H. ligurriens* and *Hy. violacea*. After 24 hours, the abdomen of rat carcasses started to inflate which marked the second stage of the decomposition process: the bloated stage. The putrid smell that was emitted from decomposed carcasses in the control group and T1 were intensified which attracted flies; *Ch. megacephala, Ch. rufifacies, Hy. violacea* (Diptera: Calliphoridae) and *Sarcophaga* sp. (Diptera: Sarcophagidae). The flies mostly gathered at the head area especially at the natural orifices, such as the mouth, nose, eyes and ears. During this time, fly eggs can be observed around the head area in between the fur. On the other hand, the decaying smell emanating from T2 carcasses was subtle and attracted a smaller number of similar flies' species.

The active decay stage started when the carcasses' abdomen starts to deflate and first instar larvae were seen to infest on the decomposed tissue of the carcasses. For carcasses in the control and T1 groups, the larvae infestation occurred and decomposed liquid can be seen oozing out from the carcasses' natural orifices which heightened the decay smell and eventually attracted more flies to the carcasses. In this stage, a pool of blowflies' larvae, which were dominated by *Ch. rufifacies* followed by *Ch. megacephala* were seen actively infesting the carcasses especially at the natural orifices such as the mouth, eyes and ears as well as at the anus area.

On the other hand, there is no oviposition observed on T2 carcasses, hence no larvae were infesting the carcasses. The decomposed body fluid can be seen slowly oozing out from the natural orifices surrounding the carcasses. The body fluid was a mixture of decomposed internal organs and blood which appear reddish and black. The pungent smell was stronger near the carcasses. Adult flies seen around the carcasses were mainly *Ch. rufifacies, Ch. megacephala* and *Sarcophaga* sp. However, no eggs were deposited, rather than several dead *Ch. rufifacies* and *Ch. megacephala* were discovered on carcasses especially in the eye socket

and abdomen area. The advance decay stage for the control group and T1 began when the L3 larvae started leaving the carcasses (5-6th day). The larvae in this stage, were seen crawling to a drier ground, away from the carcasses, and some of them digging the soil underneath the carcasses to pupate. During this stage, the carcasses were left with cartilage, skin, bones and some portion of fur. The pungent smell of the carcasses was also reduced. The flies colonising the carcasses during the earlier stage of decomposition were replaced by other insects such as beetles and ants.

As for T2 carcasses, the advanced decay stage started around the 13-14th day. During this stage, the carcass parts left was skin and bones. The fur was mostly gone and the smell was less pungent. The number of flies visiting was reduced with only one or two muscid flies can be seen. The beetles were also observed on the carcasses. Besides these insects, the fungus can be seen growing on the carcass' skin. A similar physical appearance of carcasses was observed until 2-3 days later. The differentiation between decay and advanced-decay stage was challenging due to the much slower rate of decomposition as the result of a lack of insect activity on carcasses. After a few days, the carcasses were dried (dry remains stage) and left with bones, and some of them still had small portion of fur. During this stage, no insects were observed, which indicates the end of the decomposition process. The insect succession pattern in all groups was presented in Table 5.

The mean decomposition period of each group was subjected to statistical analysis to compare the decomposition period of each group across five trials of experiments. From multivariate analysis of variance (MANOVA), there is a significant variations of decomposition period of carcasses in between trials of experiments [$F_{12, 34.69} = 7.622$, $P<0.05$; Wilk's $\Lambda=0.033$, partial $\eta^2=0680$]. Multiple comparison analysis shows a significantly longer decomposition period for all groups during the fifth trial of experiments ($P<0.05$).

In addition, there is a significant difference between control and organophosphate-intoxicated carcasses [$F_{8,108} = 26.21$, $P<0.05$; Wilk's $\Lambda = 0.116$, partial $\eta^2 = 0.66$]. Further multiple comparison analysis (Tukey HSD test) shows the decomposition of carcasses treated with chlorpyrifos was significantly longer in active decay (8.00±1.71) and advanced decay (2.85±0.93) stages ($P<0.05$compared to control and glyphosate treated carcasses.

Table 5. Insect succession pattern of rat carcasses in different groups

Order	Family	Genus/Species	Control					*T1					*T2				
			*F	B	D	AD	DR	F	B	D	AD	DR	F	B	D	AD	DR
Diptera	Calliphoridae	*Chrysomya rufifacies*	■	■	■	■		■	■	■	■		■	■	■	■	
		Chrysomya megacephala	■	■	■			■	■	■	■		■	■	■	■	
		Hemipyrellia ligurriens			■	■			■	■					■		
		Lucilia cuprina			■												
		Lucilia sp.		■													
		Hypopygiopsis violacea	■	■	■				■	■							
	Sarcophagidae	*Sarcophaga* sp.		■	■				■	■	■			■	■	■	
	Muscidae	*Hydrotea chalcogaster*				■	■			■	■	■		■	■	■	■
		Musca domestica				■	■			■	■	■		■	■	■	■
		Atherigona orientalis				■	■			■	■				■	■	

*F= Fresh, B= Bloated, D= Active decay, AD= Advanced decay, DR= Dry remains
*T1= glyphosate-intoxicated carcasses group
*T2 = chlorpyrifos-intoxicated carcasses group

DISCUSSION

Influence of Environment Parameters on Carrion Decomposition and Insect Activity

In forensic entomology, the environmental parameters are the most important factor that can influence the rate of decomposition and insect activity on cadavers and animal carrion (Mahat et al. 2009). Temperature can increase the chemical reaction inside the carcasses and also accelerate the necrophagous insects' growth which feed on the carcass' tissues (Michaud & Moreau 2009). In four-season country, the extreme changes in weather altered the rate of carcass decomposition and insects' arrival. For example, a study in Nebraska, USA, carcass decomposition during summer was three to seven times faster compared to carcass decomposition during wintertime (Meyer et al. 2013).

Malaysia on the other hand, has a humid weather throughout the year and usually influenced by the winds blowing from the Indian Ocean (Southwest monsoon) around May to September and the South China Sea (North-Eastern monsoon) around November to March (Malaysia Meteorological Department 2023). There is no significant difference of temperature and humidity during five trials of experiments, however, the rate of carcass decomposition is significantly longer in all groups during the fifth trial of experiment (November 2021) compared to decomposition period in other trials. Variation of decomposition period in between trials might be due to the continuous rain occurred during decomposition period which affects the presence of insects on carcasses, thus prolonged the time for the carcasses to reach dry stage. Previous study on the effects of weather on insect activities and decomposition period had observed there is variations in insect visiting and activities on carcasses following rain (Griffiths et al. 2020).

The minPMI estimation was based on the developmental rates of blowflies and successional patterns of various species of carrion insects' communities. Rainfall may interfere with the blowfly developmental rates by hindering the oviposition to occur on the carcass as the insect's access was limited (Ngoen-Klan et al. 2011). However, in this study, the oviposition already occurred before rain, and thus the larvae already hatched from the eggs and infested the decayed tissues. The larvae hid inside the carcass during heavy rainfall. Furthermore, light showers did not affect fly insects to oviposit. Therefore, the changes in the environment parameters in this study does not influence the duration of carcass decomposition and arrival of carrion insects significantly.

Insect activity plays an important role during decomposition process (Abd El-Bar & Sawaby 2011). In present study, chlorpyrifos-intoxicated carcasses (T2) showed a significantly longer decomposition duration ($P<0.05$) which most likely due to the absence of necrophagous larvae that are supposedly infesting on the carcasses' decomposed tissues. In previous study on insect exclusion on pig decomposition had shown the delayed access of insects to carrion has retards the decomposition rate of the carcass (Voss et al. 2008). Decomposed carrion normally released strong decay odor consists of ammonia and sulphur containing compounds which attracts gravid flies to oviposit on the carrion (Abd El-Bar & Sawaby 2011). In this study, decay odor in T2 carcasses was subtle and in addition with toxic environment, may have repelled the flies from ovipositing on the carrion tissues. Other studies have observed the presence of eggs on carcasses treated with insecticides, although delayed oviposition was observed. A study using malathion (organophosphate insecticide) intoxicated carcasses had shown delayed in oviposition and prolonged fly development of both *Ch. rufifacies* and *Ch. megacephala* (Mahat et al. 2009). In a separate study, delay of oviposition was observed although eggs' eclosion was not observed on carcass that was sprayed with insecticide (Denis et al. 2018). Delayed in fly

oviposition was also reported previously on insecticide-exposed flies (Dad & Yousuf 2011; Denis et al. 2018; Gupta et al. 2007; Mahat et al. 2009).

Dipteran Diversity Associated with Decomposed Rat Carcasses

In this present study, there is no significant difference of dipteran flies' diversity in between groups of carcasses and *Chrysomya* sp. was the most predominant fly associated with rat carcass decomposition. *Chrysomya* sp. have been reported in several studies where they were among the earliest visitors and main decomposers of carrion in Malaysia (Adrus & Rahim 2018; Azwandi et al. 2013; Chin et al. 2007). Study of fly species composition recovered from 34 human remains that were referred to Universiti Kebangsaan Malaysia Medical Centre (UKMMC) showed that the highest number of individual maggots' presence was *Ch. megacephala* (70.6%) followed by *Ch. rufifacies* (44.1%) (Syamsa et al. 2017). Besides human remains, other studies using animal carcasses have reported that both *Ch. rufifacies* and *Ch. megacephala* were the most abundant calliphorid flies visiting and infesting carcasses in peninsular Malaysia (Azwandi et al. 2013; Chin et al. 2007). Similarly, in Sarawak, the occurrence of *Ch. megacephala* and *Ch. rufifacies* as two dominant species infesting on carcasses were also observed in studies conducted in two different locations, which are; mangrove forest in Kuching and peat swamp in Kota Samarahan (Adrus & Rahim 2018; Maramat & Rahim 2015).

In this study, other flies such as flesh fly (Order: Sarcophagidae) and house fly (order: Muscidae) can also be observed on the carcasses in both the control and organophosphate intoxicated groups. The *Sarcophaga* sp. was observed during the bloated stage, which in agreement with previous studies where *Sarcophaga* sp. was found to be the earliest species to colonise a carrion in peninsular Malaysia and Egypt (Abd El-Gawad et al. 2019; Zuha et al. 2017). Meanwhile, the most common muscid flies present on carcasses was *Hydrotea chalcogaster* which was observed during the decay stage, similar to observation from other studies (Carvalho et al. 2008; Tantawi et al. 1996).

The Effects of Organophosphate Pesticides on Carcasses' Decomposition and Dipteran Succession

In this present study, the organophosphate pesticides were orally administered to the rats to mimic the pesticide poisoning that usually occur by ingestion (Kamaruzaman et al. 2020). Five decomposition stages were determined which are, fresh, bloated, active decay, advanced decay and dry remains following criteria described by Payne (1965). The decomposition duration of insecticide-intoxicated carcasses was significantly longer ($P<0.05$) compared to the control group which similar to few reports on pesticide-treated carcasses where a distinct delay in decomposition occurred when the carrion was treated with pirimiphosmethyl (Abd El-Bar & Sawaby 2011), pyrethrins and pyrethroids (de Souza Sandoval & de Ascenção Medeiros 2013) and carbamate (de Siqueira et al. 2015).

On the other hand, a study by Jales et al. (2020), administering terbufos (organophosphate) to rat carcasses has speeds up the decomposition process by 24 hours. It was detailed that the composition of terbufos has increased the metabolism of decomposing microorganisms, which raised the cellular respiration and ammonia production, hence increasing the decomposition rate of rat carcasses (Cycoń & Piotrowska-Seget 2016; Jales et al. 2020). Pechal et al. (2014) has conducted an experiment where hindered insect access to carrion has caused longer decomposition time as seen in chlorpyrifos-intoxicated (T2) carcasses in this study. The lack of flies' abundance in T2 carcasses might be due to chlorpyrifos, masking the decay odor making the carcass less attractive to the necrophagous

flies (Voss et al. 2009). However, despite the least number of flies visiting T2 carcasses, the pattern of flies observed on the carrion were similar according to the stages of decomposition except for the absence of fly immatures on the carcasses.

Meanwhile, for T1 group, the presence of glyphosate does not repel the arrival of flies. Similar colonising flies were observed on the carcasses relative to the control group. The observation was similar to previous study conducted in Kota Samarahan where the insect colonisation and duration of decomposition process of rabbit carcasses intoxicated with paraquat dichloride was similar with the control carcasses, which took about a week (Lawai et al. 2015). Paraquat and glyphosate were both herbicides, non-toxic to insects and does not mask the smell of the decay carcasses. Therefore, they do not affect the flies' arrival time and decomposition duration of the carcasses.

CONCLUSION

There is a significant difference in decomposition period of carcasses between control and chlorpyrifos-intoxicated carcasses group whereby, the later took a significantly longer to decomposed (15.8±2.82 days) following the absence of necrophagous larvae on carcasses. There is no significant difference on dipteran diversity among the three groups of carcasses and the flies succession pattern of rat carcasses is similar, although the number of individual flies' species visiting were varied. The forensic entomological baseline data in Sarawak remained lacking as well as the baseline data for the influences of organophosphate pesticides on decomposition and forensically important flies. Therefore, this report may be useful in estimating PMI in Sarawak, especially whenever the presence of organophosphate is suspected.

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AUTHORS DECLARATIONS

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

Ethics Declarations
Animal ethics approval for this research was obtained from UNIMAS Animal Ethics Committee (UNIMAS/AEC/F07/051).

Data Availability Statement
Data available upon request to corresponding author.

Authors' Contributions
NAAR and ZN design the research experiment and prepared the proposal. MO conducted the experiment including data collection and analysis, specimen identification and preparation of the manuscript. MA confirms the species identified. NAAR and MO read and finalised the manuscript.

REFERENCES

Abd El-bar, M.M. & Sawaby, R.F. 2011. A preliminary investigation of insect colonization and succession on remains of rabbits treated with an organophosphate insecticide in El-Qalyubiya Governorate of Egypt. *Forensic Science International* 208(1-3): e26-e30.

Abd El-Gawad, A., Badawy, R.M., Abd El-Bar, M.M. & Kenawy, M.A. 2019. Successive waves of dipteran flies attracted to warfarin-intoxicated rabbit carcasses in Cairo, Egypt. *The Journal of Basic and Applied Zoology* 80: 1-10.

Adrus, M. & Rahim, N. A. A. 2018. Insect succession and decomposition patterns of carcasses in Sarawak, Malaysian Borneo. *Journal of Sustainability Science and Management* 13(2): 69-84.

Anderson, G.S. & VanLaerhoven, S.L. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Science* 41(4): 617-625.

Azwandi, A., Nina Keterina, H., Owen, L.C., Nurizzati, M.D. & Omar, B. 2013. Adult carrion arthropod community in a tropical rainforest of Malaysia: analysis on three common forensic entomology animal models. *Tropical Biomedicine* 30(3): 481-494.

Bourel, B., Hédouin, V., Martin-Bouyer, L., Bécart, A., Tournel, G., Deveaux, M. & Gosset, D. 1999. Effects of morphine in decomposing bodies on the development of *Lucilia sericata* (Diptera: Calliphoridae). *Journal of Forensic Science* 44(2): 354-358.

Carvalho, C.J.B.D. & Mello-Patiu, C.A.D. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia* 52(3): 390-406.

Carvalho, L.M.L., Linhares, A.X., Trigo, J.R. 2001. Determination of drugs levels and effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. *Forensic Science International* 120: 140-144.

Catts, E.P. & Goff, M.L. 1992. Forensic entomology in criminal investigations. *Annual Review of Entomology* 37(1): 253-272.

Chin, H.C., Marwi, M.A., Salleh, A.F.M., Jeffery, J. & Omar, B. 2007. A preliminary study of insect succession on a pig carcass in a palm oil plantation in Malaysia. *Tropical Biomedicine* 24(2): 23-27.

Clearwater, J.R. 1981. Practical identification of the females of five species of *Atherigona rondani* (Diptera, Muscidae) in Kenya. *International Journal of Pest Management* 27(3): 303-312.

Conour, L.A., Murray, K.A. & Brown, M.J., 2006. Preparation of animals for research—issues to consider for rodents and rabbits. *ILAR journal* 47(4): 283-293.

Cycoń, M. & Piotrowska-Seget, Z. 2016. Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review. *Frontiers in Microbiology* 7: 1463.

Dad, I. &Yousuf, M.J. 2011. Determination of LC 50 of chlorpyrifos and neem extract on third instar larvae of house flies and their effect on fecundity. *Journal of Basic & Applied Sciences* 7(2): 169-174.

de Siqueira, A., Salvagni, F.A., Yoshida, A.S., Gonçalves-Junior, V., Calefi, A.S., Fukushima, A.R., de Souza Spinosa, H. & Maiorka, P.C. 2015. Poisoning of cats and dogs by the carbamate pesticides aldicarb and carbofuran. *Research in Veterinary Science* 102: 142-149.

de Souza Sandoval, R.C. & de Ascenção Medeiros, L.R. 2013. Influence of insecticide on insect fauna of forensic importance in rat carcasses (*Rattus norvegicus*) exposed in a suburban area near Atlantic rainforest fragments of southeastern Brazil. *Revista Brasileira de Zoociências* 15(1, 2, 3): 165-170

Denis, C.I., Nordin, N.H., Azman, A.R., Wahab, A., Ismail, D., Omar, B. & Mahat, N.A. 2018. Influence of citronella and chlorpyrifos on *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) infesting rabbit carcasses. *Tropical Biomedicine* 35: 755-768.

Dupont, F.Y.F., Champlain, D.L., Cyrille, A.A.J. & Fe´lix, B.B.C. 2011. A preliminary study of arthropod associated with carrion in Yaounde, Cameroon: A first step in forensic entomology in Central Africa. *Journal of Ecology and the Natural Environment* 3: 215–220.

Goff, M.L. & Lord, W.D. 1994. Entomotoxicology. A new area for forensic investigation. *The American Journal of Forensic Medicine and Pathology* 15(1): 51-57.

Goff, M.L. 2009. Early post-mortem changes and stages of decomposition in exposed cadavers. *Experimental and Applied Acarology* 49(1): 21-36.

Griffiths, K., Krosch, M.N. and Wright, K., 2020. Variation in decomposition stages and carrion insect succession in a dry tropical climate and its effect on estimating postmortem interval. *Forensic Sciences Research* 5(4): 327-335.

Google Map. 2023.Google Map. https://www.google.com.my/maps [4 January 2023].

Gupta, S.C., Siddique, H.R., Mathur, N., Mishra, R.K., Saxena, D.K. & Chowdhuri, D.K. 2007. Adverse effect of organophosphate compounds, dichlorvos and chlorpyrifos in the reproductive tissues of transgenic *Drosophila melanogaster*: 70 kDa heat shock protein as a marker of cellular damage. *Toxicology* 238(1): 1-14.

Heo, C.C., Latif, B., Silahuddin, S.A., Nazni, W.A. & Omar, B., 2015. Morphological descriptions on the larvae of *Hypopygiopsis fumipennis* (Walker, 1856) (Diptera: Calliphoridae). *Tropical Biomedicine* 32(1): 151-159.

Jales, J.T., de Moura Barbosa, T., Dos Santos, L.C., Rachetti, V.D.P.S. & Gama, R.A. 2020. Carrion decomposition and assemblage of necrophagous dipterans associated with terbufos (Organophosphate) intoxicated rat carcasses. *Acta Tropica* 212: 105652.

Kamaruzaman, N.A., Leong, Y.H., Jaafar, M.H., Khan, H.R.M., Rani, N.A.A., Razali, M.F., & Majid, M.I.A. 2020. Epidemiology and risk factors of pesticide poisoning in Malaysia: A retrospective analysis by the National Poison Centre (NPC) from 2006 to 2015. *British Medical Journal Open* 10(6): e036048.

Lawai, V., Abdul Rahim, N.A. & Ngaini, Z. 2015. Blowfly larval tissues as a secondary detector for determining paraquat-related death in rabbit carcass. *Journal of Forensic Sciences* 60(6): 1620-1624.

Mahat, N.A., Zafarina, Z. & Jayaprakash, P.T. 2009. Influence of rain and malathion on the oviposition and development of blowflies (Diptera: Calliphoridae) infesting rabbit carcasses in Kelantan, Malaysia. *Forensic Science International* 192(1-3): 19-28.

Malaysia Meteorological Department (MET). 2023. Weather Phenomenon. https://www.met.gov.my/en/pendidikan/fenomena-cuaca/ [10 February 2023]

Maramat, R. & Rahim, N.A.A. 2015. Forensically important flies associated with decomposing rabbit carcasses in mangrove forests in Kuching, Sarawak, Malaysia. *Forensic Science* 6(1): 79-83.

Meyer, J., Anderson, B. & Carter, D.O. 2013. Seasonal variation of carcass decomposition and gravesoil chemistry in a cold (Dfa) climate. *Journal of Forensic Sciences* 58(5): 1175-1182.

Michaud, J.P. & Moreau, G. 2009. Predicting the visitation of carcasses by carrion-related insects under different rates of degree-day accumulation. *Forensic Science International* 185(1-3): 78-83.

Nazni, W.A., Jeffrey, J., Heo., C.C., Chew, W.K. & Lee, H.L. 2011. *Bulletin No. 25*: *Illustrated Keys to Adult Flies of Forensic Importance in Malaysia.* Kuala Lumpur: Institute for Medical Research

Ngoen-Klan, R., Moophayak, K., Klong-Klaew, T., Irvine, K.N., Sukontason, K.L., Prangkio, C., Somboon, P. & Sukontason, K. 2011. Do climatic and physical factors affect populations of the blow fly *Chrysomya megacephala* and house fly *Musca domestica*? *Parasitology Research* 109: 1279-1292.

Oliveira-Costa, J. & Mello-Patiu, C.A. 2004. Application of forensic entomology to estimate the postmortem interval (PMI) in homicide investigations by Rio de Janeiro Police Department in Brazil. *Aggrawal's Internet Journal of Forensic Medicine and Toxicology* 5: 40–44

Payne, J.A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* 46: 592–602.

Pechal, J.L., Benbow, M.E., Crippen, T.L., Tarone, A.M. & Tomberlin, J.K. 2014. Delayed insect access alters carrion decomposition and necrophagous insect community assembly. *Ecosphere* 5(4): 1-21.

Rumiza, A.R., Khairul, O., Zuha, R.M. & Heo, C.C. 2010. An observation on the decomposition process of gasoline-ingested monkey carcasses in a secondary forest in Malaysia. *Tropical Biomedicine* 27(3): 373-383.

Sungur, M. & Guven, M. 2001. Intensive care management of organophosphate insecticide poisoning. *Critical Care* 5(4): 1-5.

Syamsa, R.A., Omar, B., Ahmad, F.M.S. & Hidayatulfathi, O. 2017. Comparative fly species composition on indoor and outdoor forensic cases in Malaysia. *Journal of Forensic and Legal Medicine* 45: 41-46.

Tabor, K.L., Fell, R.D., Brewster, C.C., Pelzer, K. & Behonick, G.S. 2005. Effects of antemortem ingestion of ethanol on insect successional patterns and development of *Phormia regina* (Diptera: Calliphoridae). *Journal of Medical Entomology* 42(3): 481-489.

Tantawi, T.I., El-Kady, E.M., Greenberg, B. & El-Ghaffar, H.A. 1996. Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* 33(4): 566-580.

Voss, S.C., Spafford, H. & Dadour, I.R. 2009. Hymenopteran parasitoids of forensic importance: host associations, seasonality, and prevalence of parasitoids of carrion flies in Western Australia. *Journal of Medical Entomology* 46(5): 1210-1219.

Wolff, M., Builes, A., Zapata, G., Morales, G. & Benecke, M. 2004. Detection of parathion (O, O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellín, Colombia. *Annual Aggrawal's Internet Journal of Forensic Medicine and Toxicology* 5: 6-11.

Zuha, R.M., Disney, R.H.L. & Omar, B. 2017. Scuttle flies (Diptera: Phoridae) inhabiting rabbit carcasses confined to plastic waste bins in Malaysia include new records and an undescribed species. *Tropical Life Sciences Research* 28(1): 131.

Zuha, R.M. & Disney, R.H.L. 2023. New records of three closely identical species of *Megaselia rondani* (Diptera: Phoridae) from Bangi, Selangor, Peninsular Malaysia. *Serangga* 28(1): 41-54.