Journal of King Saud University – Science 34 (2022) 101676

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Effect of the sedative drug zolpidem tartrate on the immature and mature stages of carrion flies *Chrysomya rufifacies* and *Chrysomya indiana*

Lamya Ahmed Al-Keridis [a], Fahd A. Al-Mekhlafi [b,*], Fahd Mohammed Abd Al Galil [c], Rania Ali El Hadi Mohamed [a,d], Laila A. Al-Shuraym [a], Sadeq K. Alhag [e], Muhammad A. Wadaan [b], Mohammed S. Al-Khalifa [f], Sureshchandra Popat Zambare [j]

[a] *Biology Department, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia*
[b] *Bioproducts Research Chair, Zoology Department, Faculty of Science, King Saud University, Saudi Arabia*
[c] *Department of Biology, Faculty of Sciences, University of Bisha, P.O. Box 551, Bisha 61922, Saudi Arabia*
[d] *Scientific Researcher and Research Coordinator, Federal Ministry of Health, Sudan*
[e] *Biology Department, College of Science and Arts, King Khalid University, Muhayl Asser, Saudi Arabia*
[f] *Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[j] *Zoology Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, MS, India*

ARTICLE INFO

Article history:
Received 17 April 2021
Revised 6 October 2021
Accepted 23 October 2021
Available online 27 October 2021

Keywords:
Calliphoridae
Zolpidem Tartrate
Carrion flies
Entomotoxicology

ABSTRACT

In forensic investigations many types of evidence are collected, such as urine, blood, and gastric contents. Carrion insects may also be recovered. Entomotoxicology is an approach to detecting, identifying, and quantifying chemicals in an investigation involving a death. Entomotoxicology involves the toxicological analysis of the tissues of insects recovered from a corpse. Cadaveric flies ingest chemicals contaminating the tissue and fluid on which they feed, affecting their physiology, growth, and development. We evaluated the effect of Zolfresh (zolpidem tartrate) on the development rate and growth parameters of two Calliphorid flies, *Chrysomya rufifacies* (Macquart, 1842) and *Chrysomya indiana* (Abd Algalil & Zambare, 2016). The two species of Calliphoridae were fed with fresh chopped liver treated with various concentrations of Zolfresh. We evaluated the way in which the chemical affected the morphological parameters and developmental rates of *C. indiana* and *C. rufifacies.* The rate of development in the flies was negatively correlated with the concentration of Zolfresh. The results of the study concurred with those of several studies on the same and different species of Calliphoridae. The experimental data were used to estimate insect-based postmortem interval values. In real forensic examination, this estimation can be compromised by prior use of drugs.

1. Introduction

Forensic medical investigation of death involves the analysis of samples such as urine, blood, and gastric contents. Collecting these samples from decaying bodies can be challenging. Arthropod evidence can be critical in such cases, especially when investigating suspected poisoning (Goff et al., 1988). Entomotoxicology entails the qualitative and quantitative evaluation of toxicological substances in arthropod witnesses of death, and has become widely accepted in medico-legal death investigations (Chophi et al., 2019). Insects provide an alternative source of toxicological samples that can aid the identification of the cause and time of death. The larvae of carrion flies feed on dead, decaying, or living animal tissues. Carrion flies colonize cadavers in succession (El-Kady et al., 1994). Flies of the family Calliphoridae, that feed on fresh flesh, were reported as the first visitors to a scene immediately after a death (Early and Goff, 1986). In poisoning cases, the chemicals that caused death in the victim can be transferred into the calliphorid's tissues as they feed on the intoxicated body.

Various growth and developmental stages in arthropods are affected by the chemicals ingested with their cadaveric meals. Recent studies (Goff et al., 1988) have identified chemicals in some cadaveric flies' feeding stages. Other researchers have established

* Corresponding author.
E-mail address: falmekhafi@ksu.edu.sa (F.A. Al-Mekhlafi).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101676
1018-3647/

an association between these chemicals, variations in the arthropods' life cycle, and the estimation of MPI. Several studies have confirmed the effects of different chemicals on the development of insects and the estimation of PMI. (Boon et al., 2008) found that malathion decreases the growth rate of carrion flies. (Bourel et al., 2001) confirmed the effects of insects' consumption of opiates and morphine. (Leclercq and Vaillant, 1992) investigated the effects of exposing carrion flies to lead arsenate contaminated cadavers. This research established that cadaveric flies recovered at the scene are worth investigation, in order to detect, identify, and quantify chemicals associated with the individual's death (Chophi et al., 2019). This broad applicability explains why entomotoxicology is highly valued in medico-legal death investigations.

In the current study we evaluated the effects of zolpidem tartrate (Zolfresh) on growth, as reflected by changes in morphological parameters, and developmental rate of the Calliphoridae *C. rififacies* and *C. indiana.* Zolfresh is a sedative-hypnotic medicinal substance used for treating insomnia and anxiety. Among other studies, (Darke et al., 2012; Ben-Hamou et al., 2011) linked Zolfresh to cases of suicide.

2. Materials and methods

2.1. Sample collection and identification

The calliphorid flies *C. rufifacies,* and *C indiana* were collected from Aurangabad City, Maharashtra State, India using decaying meat and a dead goat as a trap. The arthropods were collected using a standard method (Cooper and Cooper, 2013), with modifications proposed by (Abd Algalil et al., 2017). Adults were collected using EISCO Aluminum Insect Collecting Net, 30″ handle length, 12″ diameter, and forceps, as well as other insect collecting tools (Bala and Singh, 2009). The collected Calliphoridae flies were reared in separate cages sized 45″ long * 30″ high * 30″ wide. Separate cages were covered with muslin cloth. Each cage was provided with two Petri dishes, one containing cotton wetted by water sweetened with honey, and the other containing chopped fresh buffalo liver. The Calliphoridae larvae were separated morphologically, and the hairy maggots were cultured separately in beakers inside cages similar to those of the adults. The larvae were fed with chopped liver until they reached pupation. The pupae were kept in new adult rearing cages adult emergence.

The cages containing adult were screened for egg collection. Eggs were collected immediately from individual females for preparing pure cultures. The pure cultures were reared for five generations, to ensure the production of an adequate number of larvae for further experiments.

Larvae of different species were dissected under a stereo zoom microscope at 20X and 40X magnification (ERMA optical works, No. 44883, Tokyo). The dissected parts were evaluated microscopically using an MLX-DX Magnus Trinocular Microscope (Olympus PVT limited, NO 4B525145 India). Morphological identification was performed according to previously published characteristics (Abd Algalil et al., 2017; Sukontason et al., 2004). The samples were identified as *Chrysomya indiana* and *Chrysomya rufifacies.*

2.2. Experimental design

We used the sedative drug Zolfresh, produced by Indian Acme Formulation Pvt. Ltd. A 1 g/mL Zolfresh solution was prepared by dissolving a 5 mg tablet in 5.0 mL distilled water to prepare 1 mg/ml solution. From the prepared solution, different volumes (0.05 mL, 0.1 mL, 0.15 mL, and 0.20 mL) were taken and mixed with 50 gm of minced fresh liver tissue to prepare food containing 1 ppm, 2 ppm 3 ppm, and 4 ppm of zolpidem. These treated foods were kept in four cleaned beakers, and the fifth baker was kept as a control, containing 50 gms of untreated minced liver. Simultaneously, about 80–90 first instar maggots were released in the treated and untreated media to minimize the overcrowding of the larvae within the containers. Each developmental stages were monitored and recorded, to estimate the developmental duration of each stage. The experiments were carried out at the same room temperature and relative humidity (Fig. 1), with a 12 h dark/12 h light cycle.

The maggots were dipped in boiling water (Adams and Hall, 2003) to perform the morphological analysis at full relaxation. The length and width, while the weight was measured (Richards et al., 2013; Rosilawati et al., 2014; Tantawi and Greenberg, 1993).

2.3. Statistical analysis

Statistical analysis was conducted using SPSS 26 software (SPSS Inc., Chicago, IL). ANOVA tests with Tukey *post-hoc* tests were used to evaluate differences between groups. $P < 0.001$ was considered to indicate statistical significance.

3. Results

3.1. Effects of Zolfresh on the developmental rate of Chrysomya rufifacies and Chrysomya indiana

Zolpidem tartrate (Zolfresh) had a concentration-dependent effect on the development of *C. rufifacies* and *C. indiana* throughout both the feeding and the post-feeding stages of the life cycle. In the

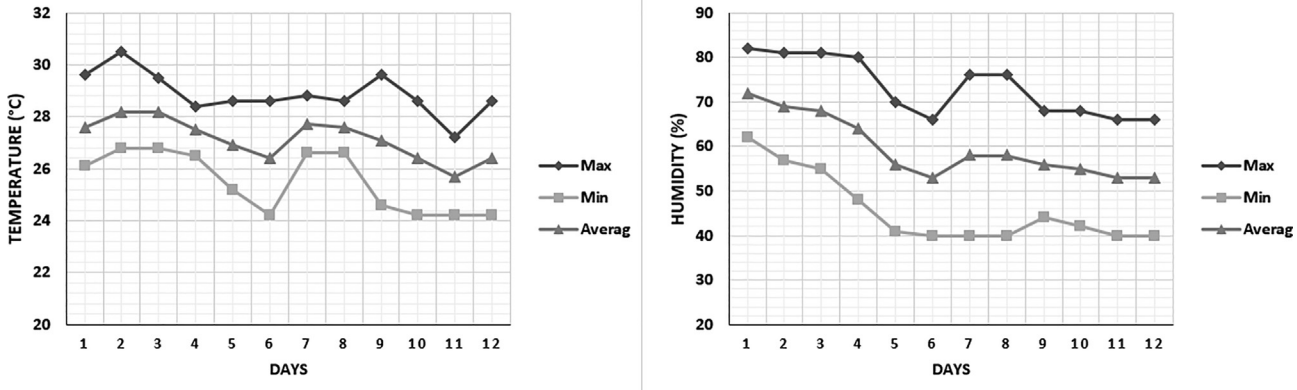

Fig. 1. Summary of temperature and humidity data obtained during the study.

Table 1
Duration (h) of the different developmental stages of *C. rufifacies* and *C. indiana* reared on various concentrations of zolpidem.

Concentration (ppm)	Insect	Duration of first instar	Duration of second instar	Duration of third instar	Duration of prepupae	Duration of pupae	Total development duration (PMI)
0	*C. rufifacies*	21.00 ± 0.00 g	24.67 ± 0.33 e	33.33 ± 0.33f	35.00 ± 0.00 d	147.67 ± 0.33d	261.67 ± 0.67f
	C. indiana	21.67 ± 0.33 eg	23.33 ± 0.33 e	30.00 ± 0.00 g	34.33 ± 0.33 d	107.67 ± 0.33i	217.00 ± 0.58 h
1	*C. rufifacies*	23.67 ± 0.33 de	27.33 ± 0.33 d	38.67 ± 0.33 e	41.33 ± 0.33c	135.33 ± 0.33e	266.33 ± 0.67 ef
	C. indiana	25.67 ± 0.33 cd	24.33 ± 0.33 e	36.67 ± 0.33 e	41.33 ± 0.33c	116.00 ± 0.58 h	244.00 ± 1.53 g
2	*C. rufifacies*	25.67 ± 0.33 cd	29.67 ± 0.33c	42.33 ± 0.33 cd	42.67 ± 0.33 bc	156.67 ± 0.33c	297.00 ± 1.00c
	C. indiana	28.00 ± 0.00 bc	29.00 ± 0.00 cd	37.67 ± 0.33 e	43.00 ± 0.00 bc	122.33 ± 0.33 g	260.00 ± 0.58f
3	*C. rufifacies*	28.00 ± 0.00 bc	32.00 ± 0.00b	44.33 ± 0.33 bc	44.33 ± 0.33b	164.33 ± 0.33b	313.00 ± 0.58b
	C. indiana	28.67 ± 0.33 ab	30.33 ± 0.33 bc	41.67 ± 0.33 d	44.67 ± 0.33b	124.66 ± 0.33 fg	270.00 ± 1.15 e
4	*C. rufifacies*	28.67 ± 0.33 ab	34.67 ± 0.33 a	48.33 ± 0.33 a	47.33 ± 0.33 a	170.00 ± 0.58a	329.00 ± 1.15 a
	C. indiana	30.67 ± 0.67 a	35.00 ± 0.58 a	45.33 ± 0.33b	47.67 ± 0.33 a	125.33 ± 0.33f	284.00 ± 1.53 d
F		93.17	154.77	319.32	228.68	3240.40	1059.41
df		9	9	9	9	9	9

Different lowercase letters in the columns indicate significant effect of the different concentrations of zolpidem ($P \leq 0.001$).

control culture of *C. rufifacies* and *C. indiana*, the different instars took minimum developmental duration compared to the treated cultured. Likewise, among the treated cultures with different concentration of zolpidem (1,2,3,4ppm) of both flies the results shown that the developmental durations of different instars were increases positively with the concentration of the respective fly species, more details are shown clearly with F and df values at $P \leq 0.001$ (Table 1).

Similarly, the durations of the prepupae and pupae of the *C. rufifacies* and *C. indiana*, also shown increases in the treated cultures positively with the concentrations i.e the treated cultures with 4 ppm took long duration compared to the 1 ppm treated cultures of the respective species. Compared with control cultures of the different species. The prepupal and pupal durations of these two species took the minimum durations compared to the treated cultures. The F and df values are shown in (Table 1).

The total duration of the life cycle of *C. rufifacies and C. indiana* in control culture took about 261.67, 217 h. while the durations in the treated cultures varied from 266.3 h to 329.00 h in *C. rufifacies*, and 244.00 h to 284.00 h in *C. Indiana* (F = 31059.41; df = 9; $P \leq 0.001$), depending on the concentration of the zolpidem (Table 1).

3.2. Effects of Zolfresh on morphological parameters of Chrysomya rufifacies and Chrysomya indiana

The length, width and weight (morphological parameters) of *C. rufifacies* and *C. indiana* larval instars as well as prepupae and pupae were shown a negative correlation with the concentration of Zolfresh. The minimum sizes of the different developmental stages were shown in the culture treated with 4 ppm and increases when the concentration of zolpidem decreases in the cultures of both species. The effect of different concentrations of zolpidem shown significant variations on these parameters at $P \leq 0.001$ and the Fand df values were shown in detail (Tables 2, 3).

Similarly, adult *C. rufifacies* and *C. indiana* were significantly decreased in size when the concentrations of the zolpidem increased (Table 4).

Table 2
Morphological parameters of the larval stages of *C. rufifacies* and *C. indiana* treated with various concentrations of zolpidem.

Concentration (ppm)	Insect	first instar			second instar			third instar		
		Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
0	*C. rufifacies*	5.43 ± 0.03 a	2.13 ± 0.03b	9.83 ± 0.03b	9.63 ± 0.03ab	3.37 ± 0.03 ab	33.37 ± 0.09b	17.57 ± 0.03b	4.33 ± 0.03b	70.10 ± 0.21b
	C. indiana	5.60 ± 0.00 a	2.30 ± 0.00 a	11.43 ± 0.07 a	9.93 ± 0.07 a	3.53 ± 0.03 a	35.40 ± 0.12 a	19.07 ± 0.07 a	4.60 ± 0.00 a	78.60 ± 0.06 a
1	*C. rufifacies*	4.57 ± 0.03c	2.00 ± 0.00c	9.07 ± 0.07c	9.33 ± 0.03 bc	3.00 ± 0.00 cd	28.63 ± 0.07 d	14.57 ± 0.03 d	4.00 ± 0.00c	54.23 ± 0.03 d
	C. indiana	5.17 ± 0.03b	2.10 ± 0.00b	10.07 ± 0.07b	9.23 ± 0.03 d	3.20 ± 0.00 bc	31.07 ± 0.07c	15.30 ± 0.10c	4.27 ± 0.03b	58.47 ± 0.07c
2	*C. rufifacies*	4.37 ± 0.07c	1.00 ± 0.00f	8.53 ± 0.07 d	8.53 ± 0.07 e	2.53 ± 0.07 e	26.77 ± 0.03 e	13.00 ± 0.00f	3.63 ± 0.03 d	46.27 ± 0.07f
	C. indiana	4.43 ± 0.07c	1.50 ± 0.00 d	9.43 ± 0.03c	8.57 ± 0.07 e	2.87 ± 0.03 d	28.93 ± 0.07 d	14.43 ± 0.07 d	4.00 ± 0.00c	50.13 ± 0.13 e
3	*C. rufifacies*	3.63 ± 0.03 e	0.90 ± 0.00	8.07 ± 0.07 fe	7.33 ± 0.07f g	2.00 ± 0.00f	23.33 ± 0.03 g	11.13 ± 0.07 h	3.20 ± 0.00 e	41.10 ± 0.21 g
	C. indiana	4.00 ± 0.00 d	1.20 ± 0.00 e	8.43 ± 0.03 de	7.57 ± 0.03f	2.53 ± 0.03 e	27.17 ± 0.09 e	13.57 ± 0.03 e	3.67 ± 0.03 d	45.53 ± 0.03f
4	*C. rufifacies*	3.40 ± 0.00 e	0.80 ± 0.00 g	7.40 ± 0.10 g	6.60 ± 0.06 h	1.67 ± 0.03 g	20.10 ± 0.06 h	10.00 ± 0.00 i	3.00 ± 0.00f	36.77 ± 0.12 i
	C. indiana	3.50 ± 0.0 e	1.00 ± 0.00f	8.00 ± 0.00f	7.07 ± 0.07 g	2.40 ± 0.00 e	26.03 ± 0.03f	12.43 ± 0.03 g	3.30 ± 0.00 e	40.27 ± 0.03 h
F		473.529	3090.67	403.39	456.64	356.56	4191.93	2733.49	626.44	13623.71
df		9	9	9	9	9	9	9	9	9

Different lowercase letters in the columns indicate significant effect of the different concentrations of zolpidem ($P \leq 0.001$).

Table 3
Morphological parameters of prepupae of *C. rufifacies* and *C. indiana* treated with various concentrations of zolpidem.

Concentration (ppm)	Insect	prepupae			pupae		
		Length (mm)	Width (mm)	Weight(mg)	Length (mm)	Width(mm)	Weight(mg)
0	*C. rufifacies*	12.27 ± 0.09c	4.30 ± 0.00b	65.87 ± 0.07 a	9.77 ± 0.03b	4.17 ± 0.03 ab	48.93 ± 0.23b
	C. indiana	13.70 ± 0.10 a	4.53 ± 0.03 a	58.43 ± 0.12b	10.50 ± 0.00 a	4.33 ± 0.03 a	56.47 ± 0.07 a
1	*C. rufifacies*	10.93 ± 0.07 d	3.87 ± 0.03 d	54.97 ± 0.03c	9.13 ± 0.07 d	3.80 ± 0.00c	42.27 ± 0.27 d
	C. indiana	12.73 ± 0.07b	4.30 ± 0.00b	50.13 ± 0.13 d	9.43 ± 0.03c	4.00 ± 0.00b	45.27 ± 0.03c
2	*C. rufifacies*	9.53 ± 0.03f	3.73 ± 0.03 de	44.53 ± 0.03 e	8.47 ± 0.03 e	3.47 ± 0.03 d	35.30 ± 0.10f
	C. indiana	10.90 ± 0.10 d	4.07 ± 0.03c	41.10 ± 0.21f	8.70 ± 0.00 e	3.67 ± 0.03c	37.67 ± 0.07 e
3	*C. rufifacies*	8.67 ± 0.03 g	3.27 ± 0.03 g	41.40 ± 0.10f	7.57 ± 0.03 g	2.97 ± 0.03 e	32.07 ± 0.07 g
	C. indiana	10.00 ± 0.00 e	3.57 ± 0.03 ef	38.67 ± 0.12 g	8.00 ± 0.00f	3.37 ± 0.03 d	35.23 ± 0.12f
4	*C. rufifacies*	7.17 ± 0.03 h	3.00 ± 0.00 h	36.60 ± 0.00 h	6.57 ± 0.07 i	2.70 ± 0.00f	26.33 ± 0.03 h
	C. indiana	8.83 ± 0.03 g	3.43 ± 0.03 fg	33.43 ± 0.03 i	7.27 ± 0.03 h	3.00 ± 0.00 e	31.70 ± 0.06 g
F		1003.35	318.67	10221.68	1021.68	446.37	4901.36
df		9	9	9	9	9	9

Different lowercase letters in the columns indicate significant effect of the different concentrations of zolpidem ($P \leq 0.001$).

Table 4
Morphological parameters of *C. rufifacies* and *C. indiana* treated with various concentrations of zolpidem.

Concentration (ppm)	Insect	Length (mm)	Width (mm)	Weight (mg)
0	*C. rufifacies*	9.67 ± 0.03 a	4.00 ± 0.00b	42.57 ± 0.07 a
	C. indiana	9.87 ± 0.07 a	4.53 ± 0.03 a	50.73 ± 0.03 a
1	*C. rufifacies*	9.00 ± 0.00c	3.40 ± 0.00c	37.23 ± 0.03 a
	C. indiana	9.40 ± 0.00b	4.03 ± 0.03b	39.50 ± 0.00 a
2	*C. rufifacies*	8.00 ± 0.00 e	3.00 ± 0.00 d	32.53 ± 0.03 a
	C. indiana	8.50 ± 0.06 d	3.47 ± 0.03c	22.57 ± 9.68 a
3	*C. rufifacies*	7.07 ± 0.03 g	2.77 ± 0.03 e	29.27 ± 0.07 a
	C. indiana	7.57 ± 0.03f	3.00 ± 0.00 d	22.20 ± 9.35 a
4	*C. rufifacies*	6.43 ± 0.03 h	2.53 ± 0.03f	25.57 ± 0.03 a
	C. indiana	7.33 ± 0.03f	2.77 ± 0.03 e	28.20 ± 0.00 a
F		1052.27	649.72	4.81
df		9	9	9

Different lowercase letters in the columns indicate significant effect of the different concentrations of zolpidem ($P \leq 0.001$).

4. Discussion

In this study two hairy maggot species were used to evaluate the effects of different concentrations of the sedative drug Zolfresh (zolpidem tartrate) on the estimation of PMI and the morphological parameters of the carrion insects. In the treated cultures of *C. rufifacies* the PMI (the total life cycle) were delayed compared to the control culture, this delay also shown among the treated cultures with different concentration. In the 1 ppm treated culture the delay was 5 h, and in the 4 ppm culture the delay was about 68 h, while in the cultures treated with 2 ppm and 3 ppm delays were about 36 h and 52 h, respectively.

Many various studies agreed with the results obtained in the current study. (Rashid et al., 2013) investigated the effect of ketum extract on the developmental rate of *C. rufifacies* and *C. megacephala* and reported that the duration of development through the post-feeding and feeding stages was prolonged for about 62 and 42 h in *C. rufifacies* and *C. megacephala*, respectively. Also *C. ruffacies* completed development more slowly when treated with Alprazolam and Diazepam than in the control culture, and the time of development increased as the Diazepam concentration increased (Mali, 2011). Similarly the *C. megacephala, L. cuprina,* and *L. sericata* developmental duration were increases when the Diazepam concentration were increases (Pawar, 2011). (Bourel et al., 2001) found that the *Lucilia sericata* life cycle was affected when the flies were reared on rabbit carcass injected with different doses of morphine. They reported that the presence of morphine could lead to a underestimation of PMI determination by up to 24 h. (Tabor et al., 2005) also reported significant variations in PMI duration and the length among maggots of *Phormia regina* treated with varying concentrations of ethanol. Such findings are in concurrence with those of the current study.

Each species of Calliphoridae flies may respond differently to various chemical compounds, leading to acceleration or retardation of the development of calliphorids. (Patil, 2010) investigated the effect of Alprazolam and Diazepam on *C. bezziana*. The Alprazolam results were contrary to those of the current study. Patil found that control cultures without Alprazolam of *C. bezziana* could complete their life cycle in 12 days, maggots in 0.4 ppm Alprazolam spent 11 days, and both 1.2 and 1.6 ppm concentrations of Alprazolam led to a life cycle duration of 10 days. The study concluded that Alprazolam accelerates the development of *C. bezziana*. (Goff and Lord, 2001) studied the development of larvae of the flesh fly - *Boettcherisca peregrine* reared on tissue obtained from rabbits that

received varying doses of heroin before death. PMI estimates based on larval and pupal development were altered by up to 29 h and 18–38 h, respectively. They concluded that the arthropod-based estimation of PMI may be compromised by drug use prior to death. Similarly, the current study found that the estimated PMI varied in the treated culture of *C. rufifacies*, and the duration was prolonged as the concentration of the zolpidem increased. Decreases in the size of morphological features of different developmental stages as the zolpidem concentration increased. The greatest sizes were observed in 1 ppm culture among all the treated cultures, but it was smaller than the sizes of different stages in the control culture.

C. indiana shown similar results also. The minimum estimated PMI in the control culture was 217 h while in cultures treated with 1 ppm, 2 ppm, 3 ppm or 4 ppm the estimated PMIs were 244 h, 260 h, 270 h, and 284 h, so the duration was prolonged by about 27 h, 43 h, 53 h, and 67 h, respectively. When the concentration increased the duration of the life cycle also increased. These findings are in agreement with those of (Rashid et al., 2013) on the effect of extraction of ketum on the development of *Chrysomya rufifacies* at different doses, they reported that the alkaloid Mitragynine delayed the developmental rate by up to 62 h.

Similarly, the treated cultures of *C. indiana* showed variations in the morphological parameters compared to the control culture.

The most extensive developmental stage for each calliphorid species, including *C. rufifacies* and *C. indiana*, aided in estimating the minimum PMI value. These results are in agreement with a previous study on the influence of methylphenidate hydrochloride on the development of the forensically significant blowfly *Chrysomya chloropyga* in South Africa (Visser, 2016).

These chemicals may significantly disrupt the cellular physiology of carrion flies (Introna et al., 2001). Such disruptions may accelerate or retard developmental rates through various life cycle stages. They may extend or reduce the duration spent in certain developmental stages (de Carvalho, 2010).

Toxicological analysis of calliphorid arthropods collected from death scenes can lead to forensic determination of cause and time of death (Anderson et al., 2001; Babu et al., 2013). The rate of development through the various stages of an insect's life cycle, and the measurements of morphological parameters facilitates insect-based PMI estimation, as these insects colonize cadavers (Gennard, 2012). Different developmental stages of *C. rufifacies* and *C. indiana* were delayed by exposure to Zolfresh and the growth retardation associated with a declined in the morphological parameters (weight, width, and length).

Factors such as the presence of different drugs in the cadaver should be considered in any insect-based forensic investigation.

5. Conclusions

Calliphorid flies usually feed on cadavers. The sedative drug zolpidem produced adverse effects on the developmental rate and morphological parameters of treated Calliphorid species. The duration of development through the feeding and post-feeding stages of *C. rufifacies* and *C. indiana* was significantly prolonged depending on the concentration of Zolfresh. While the morphological parameters were significantly reduced in the treated cultures. The measurements facilitate the estimation of PMI and can consequently aid in determining the time of death. This research supports the extensive use of arthropod evidence in forensic death investigations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Groups Program Grant no. (RGP-1442-0036).

References

Abd Algalil, F.M.A., Zambare, S.P., Khan, L.A., Mali, K.H., 2017. Effect of seasonal temperature variations on the life cycle duration of forensically important calliphorid fly, *Chrysomya saffranea* (Bigot, 1877). J. Forensic Res. 8, 2.

Adams, Z.J.O., Hall, M.J.R., 2003. Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. Forensic Sci. Int. 138 (1-3), 50–61.

Anderson, G.S., Byrd, J., Castner, J., 2001. Insect succession on carrion and its relationship to determining time of death. Forensic Entomol. 143, 76.

Babu, B.S., Sharma, H., Bharti, M., Colony, B., 2013. Estimation of post mortem interval by rearing *Chrysomya rufifacies* (Macquart, 1842) (Diptera: Calliphoridae): A case study from central India. Anil Aggrawal J. Forensic Med. Toxicol 14, 1–11.

Bala, M., Singh, D., 2009. Effect of medium on the pupation behaviour of two species of blow flies (Diptera: Calliphoridae). J. Entomol. Res. 33 (3), 269–272.

Ben-Hamou, M., Marshall, N.S., Grunstein, R.R., Saini, B., Fois, R.A., 2011. Spontaneous adverse event reports associated with zolpidem in Australia 2001–2008. Journal of sleep research 20(4), 559-568.

Boon, P.E., Van der Voet, H., Van Raaij, M.T.M., Van Klaveren, J.D., 2008. Cumulative risk assessment of the exposure to organophosphorus and carbamate insecticides in the Dutch diet. Food Chem. Toxicol. 46 (9), 3090–3098.

Bourel, B., Tournel, G., Hedouin, V., Deveaux, M., Goff, M.L., Gosset, D., 2001. Morphine extraction in necrophagous insects remains for determining ante-mortem opiate intoxication. Forensic Sci. Int. 120 (1-2), 127–131.

Chophi, R., Sharma, S., Sharma, S., Singh, R., 2019. Forensic entomotoxicology: current concepts, trends and challenges. J. Forensic Leg. Med. 67, 28–36.

Cooper, J.E., Cooper, M.E., 2013. Wildlife Forensic Investigation: Principles and Practice. CRC Press.

Darke, S., Deady, M., Duflou, J., 2012. Toxicology and characteristics of deaths involving zolpidem in New South Wales, Australia 2001–2010. J. Forensic Sci. 57 (5), 1259–1262.

de Carvalho, L.M.L., 2010. Toxicology and forensic entomology. In: Amendt, J., Goff, M.L., Campobasso, C.P., Grassberger, M. (Eds.), Current Concepts in Forensic Entomology. Springer, pp. 163–178.

Early, M., Goff, M.L., 1986. Arthropod succession patterns in exposed carrion on the island of O'ahu, Hawaiian Islands, USA. J. Med. Entomol. 23 (5), 520–531.

El-Kady, E., Essa, Y., Shalaby, O., 1994. Variations in the blow and flesh flies succession on rabbit carrions killed by different methods. J. Egypt. Ger. Soc. Zool 13, 451–489.

Gennard, D., 2012. Forensic Entomology: An Introduction. John Wiley & Sons.

Goff, M., Lord, W.D., 2001. Entomotoxicology: Insects as toxicological indicators and the impact of drugs and toxins on insect development. Forensic entomology: The utility of arthropods in legal investigations, 331-340.

Goff, M.L., Omori, A.I., Gunatilake, K., 1988. Estimation of postmortem interval by arthropod succession. Three case studies from the Hawaiian Islands. Am. J. Forensic Med. Pathol. 9 (3), 220–225.

Introna, F., Campobasso, C.P., Goff, M.L., 2001. Entomotoxicology. Forensic Sci. Int. 120 (1-2), 42–47.

Leclercq, M., Vaillant, F., 1992. Entomologie et médecine légale: une observation inédite. Ann. Soc. Entomol. France, 3–8.

Mali, K.H., 2011. study of insects of crime investigation importance from Nandurbar.

Patil, A.J., 2010. Study on carrion fedder insects of forensic importance from Ahmednagar.

Pawar, H.M., 2011. Study on calliphorids of forensic importance from Aurangabad.

Rashid, A., Siti, A., Siti, F., Reena, A., Sharifah, H., Nurul, F., Nazni, W., 2013. Forensic implications of blowfly *Chrysomya rufifacies* (Calliphoridae: Diptera) development rates affected by ketum extract. Indian J. Med. Biomed. Eng. Pharm. 7, 70–74.

Richards, C.S., Rowlinson, C.C., Hall, M.J.R., 2013. Effects of storage temperature on the change in size of *Calliphora vicina* larvae during preservation in 80% ethanol. Int. J. Legal Med. 127 (1), 231–241.

Rosilawati, R., Baharudin, O., Syamsa, R., Lee, H., Nazni, W., 2014. Effects of preservatives and killing methods on morphological features of a forensic fly, *Chrysomya megacephala* (Fabricius, 1794) larva. Trop. Biomed. 31 (4), 785–791.
Sukontason, K., Sukontason, K.L., Ngern-Klun, R., Sripakdee, D., Piangjai, S., 2004. Differentiation of the third instar of forensically important fly species in Thailand. Ann. Entomol. Soc. Am. 97 (6), 1069–1075.
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). J. Med. Entomol. 42 (3), 481–489.
Tantawi, T., Greenberg, B., 1993. The effect of killing and preservative solutions on estimates of maggot age in forensic cases. J. Forensic Sci. 38 (3), 702–707.
Visser, H., 2016. The influence of Methylphenidate on the development of the forensically significant blow fly *Chrysomya chloropyga* (Diptera: Calliphoridae) in the Western Cape Province. University of Cape Town.