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# Using Larvae And Adults Of Different Species Of Flies To Detect Certain Homicides

**Ahmed Ibrahim Abdullah** **Saeed Maher Lafta**

1, Department of Science, College of Basic Education / Sharqat, Tikrit University

2, Department of Biology, College of Science, Tikrit University.

E1: ahmed.i.abdullah@tu.edu.iq E2: saeed.m.lafta@tu.edu.iq

***Abstract:*** *The current study was conducted at the animal house of the College of Veterinary Medicine, Tikrit University, from September 1, 2024, to January 20, 2025. The study involved three groups of rabbits, each subjected to different methods of euthanasia. The first group was administered gunpowder powder, the second group was administered a solution of tramadol, and the third group was administered a pesticide (toxin). The rabbits were then slaughtered and left for insects to access. Fly larvae of house flies, flesh flies, and blue flies were collected after 3, 6, 8, and 11 days post-mortem, while adults were collected after 13, 15, 18, 21, 25, and 27 days post-mortem. The concentrations of gunpowder, tramadol, and the toxin in the larvae and adults of the flies were measured using HPLC. The results were as follows: The blue fly showed a correlation in numerical data across the days, with concentrations of gunpowder in the larvae exceeding the holding time concentration on days 3 and 11, reaching values of 4.999 and 5.629 µL/gram, respectively. The average adult blue flies recorded the highest concentration of 5.576 µL/gram. For tramadol, the highest concentration recorded among species averages was 6.844 µL/gram. Additionally, the blue fly larvae excelled in recording the highest concentrations throughout the study period, all exceeding the standard holding time. Meanwhile, the adult blue flies recorded tramadol concentrations higher than the holding time concentration throughout the study period, which may indicate this species' ability to retain tramadol traces for a longer duration, as well as its sensitivity to tramadol and effectiveness in detecting it. The larvae of the flesh fly recorded the highest concentration of the toxin as an average over the study period of 9.040 µL/gram, while adult blue flies recorded a significant concentration above the standard holding time concentration of 8.797 µL/gram.*

***Keywords***: *Insects, homicides, forensic evidence, fly larvae, adult flies.*

## INTRODUCTION

Forensic entomology is the study of insects that inhabit the remains of humans and animals for legal purposes, assisting law enforcement agencies in solving criminal cases. It helps determine the time elapsed since death until the remains are discovered (Sharma *et al*., 2015). A new term related to the study of forensic entomology is the postmortem interval (PMI), defined as the time taken from the colonization of necrophagous insects until the last stage of their development or adulthood (Byrd and Castner, 2010). This insect timeline is utilized when traditional markers or methods for estimating postmortem intervals weaken, and the remains become decomposed or unrecognizable. Forensic entomology provides a concurrent timeline used to determine the time since death. When the time since death exceeds 72 hours, this synchronous method in the timeline of forensic entomology becomes an integral part of investigations related to death (Mona *et al*., 2019).

Although insects have been known to be used in crime detection for a long time, there has not been a significant increase in the popularity of this subject until the last thirty years. Forensic entomology is recognized in many countries as an important tool in legal investigations. Unfortunately, this field has not received much attention in some parts of the world, such as certain Arab countries, where the value of insects as indicators in criminal investigations has not been fully appreciated (AL-MESBAH, 2010).

Solving complex criminal cases involving issues of victim identification and the time elapsed since the crime occurred has been a long-standing task for crime scene scientists. Some circumstantial evidence can be highly valuable in providing timelines and excluding causes and times of death. One of these circumstantial criteria is forensic entomology. Although it is not a priority in criminal investigations, it holds significant importance in cases of unknown and unnatural deaths. Multiple factors must be considered when processing entomological data that affect postmortem intervals. A thorough study of insect life cycles, similar insect groups, and the correct and approved methods for collecting, rearing, and identifying insects can provide indicators regarding the cause, manner, time, place, and circumstances surrounding unknown or unnatural deaths (Mona *et al.*, 2019).

There are two established methods used to estimate the postmortem interval, based on larval development and the succession of insect species. Recent scientific and technological advancements in this field, such as integrating with toxic substances and forensic materials, molecular DNA-based identification of insect species, and various other methods for estimating postmortem intervals, represent numerous emerging areas. Forensic entomology has recently become very popular among forensic investigators and forensic entomologists (Singh *et al.*, 2022).

Most heinous crimes, such as robbery, murder, assassination, violence, and police confrontations, are frequently associated with the use of firearms. Consequently, firearms play a crucial role in forensic investigations regarding their evidential value. Firearm-related evidence assists in gathering information about the crime scene, providing various answers for forensic experts, such as: (1) whether the death resulted from homicide, accident, self-defense, or suicide, and (2) giving a brief insight into how the crime occurred (Aleksander, 2003).

High-performance liquid chromatography (HPLC) is an important technique used in forensic toxicology, which is a branch of forensic science. It involves the application of various scientific principles, methods, and techniques to aid in clinical or legal investigations related to death, poisoning, drug abuse, and the investigation of toxic substances in forensic contexts. HPLC is also vital for the detection of illicit drugs, alcohol, and common toxins. It emphasizes the importance of identifying and quantifying toxic substances in biological and non-biological samples to support evidence-based conclusions in forensic investigations (Kapoor *et al.*, 2023).

The current study aims to evaluate the efficacy of larvae and adults of three fly species (house fly *Musca domestica*, flesh fly *Calliphora vicina*, and blue fly *Lucilia sericata*) in detecting homicides involving gunpowder, drugs (tramadol), and toxins (pesticide).

## MATERIALS AND METHODS

*2.1. Field Part:* The current study was conducted at the animal house of the College of Veterinary Medicine, Tikrit University, from September 1, 2024, to January 20, 2025. The study included three groups of rabbits, each subjected to different methods of euthanasia. The first group was administered five cc of gunpowder powder directly to simulate a gunshot wound. The second group was administered five cc of a tramadol solution to simulate drug-induced euthanasia, while the third group was administered five cc of the insecticide Orio (of English origin) to simulate poisoning. The rabbits were slaughtered six hours after administration of the lethal substances to ensure the material reached the liver and entered the bloodstream; however, the third group died before reaching the six-hour mark.

Each group was then placed in a cage to protect them from predators, with the cage door opened daily for ten hours to allow targeted insects to access the carcasses, after which it was closed until the following day. The carcasses of the rabbits remained in this position until sample collection was completed. Larval samples of the studied fly species (house fly, flesh fly, and blue fly) were collected on days three, six, eight, and eleven, while adult flies were collected on days fifteen, eighteen, twenty-one, twenty-five, and twenty-seven. The insect samples were placed in plastic tubes, sealed tightly, and labeled with the sample details (insect type, developmental stage, and collection date) and stored in a freezer at -4 °C until transported to the laboratory for necessary analyses.

*2.2. Laboratory Part - High-Performance Liquid Chromatography (HPLC) Analysis:* After collecting larval and adult samples of the three-fly species under study, the concentrations of the lethal substances were examined in the laboratories of the Ministry of Science and Technology in Baghdad. An HPLC system, the Shimadzu Prominence

I LC2030 Plus (Kyoto, Japan), equipped with a Shimadzu LC 2030 UV-Vis detector, was used to separate the compounds administered to the experimental animals from the extracts of the larvae and adults of the studied fly species after they were digested using nitric acid (HNO3) and hydrogen peroxide ($H_2O_2$) in a closed-vessel microwave digestion system. The external calibration technique was used to conduct HPLC analysis under isocratic conditions. Before starting the operation in the column, the mobile phase was degassed and filtered through a membrane using methanol and 0.5% acetic acid in water (90:10 v/v). A C-18 column (4.6 × 250 mm) with a particle size of 5 micrometers was used and maintained at a temperature of 25 °C.

Each injection volume was prepared at 20 µL and then injected into the HPLC system. Samples were filtered using a 0.45-micron membrane filter (Millipore) before being placed in vials, with a flow rate of 1.0 mL/min. Spectral information was analyzed in the range of 200-400 nm, and chromatograms were detected at a wavelength of 280 nm. Based on previous results, the quantity of each component present in the insect extracts was determined. Peak identification was carried out by comparing the retention times of specific standards with those of the extract.

*2.3. Statistical Analysis:* After collecting the required data for the studied variables, it was entered into a computer and organized using Microsoft Office Excel. Statistical analysis and comparisons of the means of the concentrations of the lethal substances in the larvae and adults of the studied fly species at the specified time intervals were performed using Duncan's multiple range test with the Statistical Analysis System (SAS) (Al-Zubaidi and Al-Jubouri, 2022).

## RESULTS AND DISCUSSION

### GUNPOWDER

*3.1.1. Larvae:* The results presented in Table (1) indicate that house flies exhibited variability in numerical data across the days, suggesting a change in gunpowder concentration or a response of the larvae over time. All daily readings and their averages were lower than the holding time concentration. In contrast, flesh flies demonstrated relative stability in numerical data across the days, with the results for detecting gunpowder in the larvae exceeding the holding time concentration on days 6, 8, and 11, with values of 5.012 µL/gram for each day. This may indicate the ability of this species to retain traces of gunpowder for a longer duration.

The blue flies showed convergence in numerical data across the days, with gunpowder concentrations in the larvae exceeding the holding time concentration on days 3 and 11, with values of 4.999 and 5.629 µL/gram, respectively. The average concentration for this species was close to the standard holding time concentration, suggesting this species' sensitivity to gunpowder and its ability to detect it effectively. Each fly species may have different efficiencies in detecting gunpowder, which could be related to the life cycle of the larvae or their capacity to absorb chemical substances. Additionally, the passage of time may affect the concentration of gunpowder in the larvae, underscoring the need for immediate analyses after collecting samples from the crime scene.

Table (1): Results of gunpowder concentration in the larvae of the studied fly species at different intervals.

| Fly species | Holding time/ minute | 3 /day | 6 /day | 8 /day | 11 /day | Means of species |
|---|---|---|---|---|---|---|
| *M. domestica* | 4.641 | 2.304 i | 2.483 h | 3.656 g | 3.989 f | 3.108 c |
| *C. vicina* | 4.995 | 3.656 g | 5.012 b | 5.012 b | 5.012 b | 4.673 b |
| *L. sericata* | 4.995 | 4.999 c | 4.651 d | 4.348 e | 5.629 a | 4.907 a |

Means with the same letter are not significantly different.

*3.1.2. Adults:* The results presented in Table (2) indicate significant differences in gunpowder concentrations in the adult flies throughout the study periods. The findings for the studied fly species suggest an increase in gunpowder concentration or a response of the adults over time. The concentrations of gunpowder in adult flies exceeded the standard holding time concentration in all readings of the study, except for the blue flies on day 27, which recorded the lowest concentration of 4.982 µL/gram. The highest significant concentration was observed in flesh fly adults, reaching 6.789 µL/gram on day 27.

In terms of species averages, all concentrations exceeded the standard concentration, with the blue fly recording the highest concentration of 5.576 µL/gram. This indicates that each fly species has different efficiencies in detecting gunpowder, which may be related to the life cycle of the adults or their capacity to absorb chemical substances. Based on these results, the blue fly may be the most sensitive to gunpowder. Furthermore, the passage of time may affect the concentration of gunpowder in adult flies, highlighting the necessity for immediate analyses after collecting samples from the crime scene.

Table (2): Results of gunpowder concentration in the adults of the studied fly species at different intervals.

| Fly species | Holding time/ minute | 15 /day | 18 /day | 21 /day | 25 /day | 27 /day | Means of species |
|---|---|---|---|---|---|---|---|
| *M. domestica* | 4.641 | 4.999 m | 5.111 l | 5.616 i | 5.989 c | 6.012 b | 5.545 c |
| *C. vicina* | 4.995 | 5.121 k | 5.201 j | 5.011 m | 5.666 h | 6.789 a | 5.558 b |
| *L. sericata* | 4.995 | 5.692 f | 5.683 g | 5.738 e | 5.783 d | 4.982 n | 5.576 a |

Means with the same letter are not significantly different.

**TRAMADOL**

*3.2.1. Larvae:* The results presented in Table (3) indicate significant differences among the larvae of the studied fly species in detecting drug-related crimes based on tramadol concentrations at different intervals. The house fly exhibited variability in concentrations over the days, which may suggest a change in tramadol concentration or a response of the larvae over time. In contrast, the flesh fly showed relative stability in concentrations with time, indicating this species' ability to retain tramadol traces for a longer duration. The blue fly demonstrated convergence in concentrations throughout the study period, suggesting this species' sensitivity to tramadol and its effective detection capability, with the highest concentration recorded among species averages reaching 6.844 µL/gram.

In terms of individual concentrations, the blue fly also recorded the highest concentrations throughout the study period, all exceeding the standard holding time concentration. The house fly larvae provided concentrations higher than the standard holding time only on days 8 and 11, while all concentrations in the flesh fly larvae were below the standard holding time.

Table (3): Results of tramadol concentration in the larvae of the studied fly species at different intervals.

| Fly species | Holding time/ minute | 3 /day | 6 /day | 8 /day | 11 /day | Means of species |
|---|---|---|---|---|---|---|
| *M. domestica* | 6.588 | 4.775 h | 4.727 i | 6.601 e | 6.6 e | 5.676 b |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. vicina* | 6.588 | 4.545 j | 4.777 h | 6.102 f | 6.006 g | 5.358 c |
| *L. sericata* | 6.588 | 6.674 d | 6.784 c | 6.865 b | 7.054 a | 6.844 a |

Means with the same letter are not significantly different.

*3.2.2. Adults:* The results presented in Table (4) indicate significant differences among the adults of the studied fly species in the concentrations of tramadol administered to the experimental animals. Notably, the blue flies recorded the highest significant concentration of tramadol, reaching 7.548 µL/gram, exceeding the standard holding time concentration. The tramadol concentrations in the adults of the studied fly species ranged from 4.673 µL/gram in house flies on day 25 to 7.876 µL/gram on day 27.

For both house flies and flesh flies, the concentration of tramadol in the adults exceeded the holding time concentration on days 15 and 18, then began to decrease thereafter. In contrast, the adult blue flies-maintained tramadol concentrations higher than the holding time concentration throughout the study period. This may indicate the ability of this species to retain tramadol traces for a longer duration, as well as its sensitivity to tramadol and its effective detection capability.

Table (4): Results of tramadol concentration in the adults of the studied fly species at different intervals.

| Fly species | Holding time/ minute | 15 /day | 18 /day | 21 /day | 25 /day | 27 /day | Means of species |
|---|---|---|---|---|---|---|---|
| *M. domestica* | 6.588 | 6.731 h | 6.913 f | 5.841 k | 4.673 n | 4.977 m | 5.827 c |
| *C. vicina* | 6.588 | 6.731 h | 6.881 g | 6.011 i | 5.878 j | 5.011 l | 6.102 b |
| *L. sericata* | 6.588 | 7.104 e | 7.254 d | 7.654 c | 7.852 b | 7.876 a | 7.548 a |

Means with the same letter are not significantly different.

**TOXIN (PESTICIDE)**

*3.3.1. Larvae:* The results presented in Table (5) indicate significant differences among the larvae of the studied insect species in the concentrations of the toxin (pesticide) collected from the victims (experimental animals). The species averages show that the larvae of the flesh fly recorded the highest average concentration throughout the study period, reaching 9.040 µL/gram, exceeding the standard holding time concentration, followed by the house fly larvae with an average concentration of 8.700 µL/gram.
In terms of individual concentrations, the larvae of the flesh fly consistently recorded the highest levels throughout the study, which were above the standard holding time concentration, ranging between 8.987 and 9.132 µL/gram on days 11 and 6, respectively. This was followed by the house fly larvae, which recorded one concentration exceeding the standard holding time concentration of 8.961 µL/gram on day 8. In contrast, the concentrations of the toxin in the blue fly larvae were lower than the standard holding time concentration throughout the study period.

These results may indicate a change in toxin concentration or a response of the larvae over time. Notably, the larvae of the flesh fly demonstrated the ability to retain traces of the toxin for a longer duration.

Table (5): Results of toxin concentration in the larvae of the studied fly species at different intervals.

| Fly species | Holding time/ minute | 3 /day | 6/day | 8/day | 11/day | Means of species |
|---|---|---|---|---|---|---|
| *M. domestica* | 8.683 | 8.621 f | 8.673 e | 8.961 d | 8.546 g | 8.700 b |
| *C. vicina* | 8.683 | 9.043 b | 9.132 a | 8.999 b | 8.987 c | 9.040 a |

| | | | | | | |
|---|---|---|---|---|---|---|
| *L. sericata* | 8.683 | 7.654 j | 7.876 i | 8.218 h | 8.671 e | 8.105 c |

Means with the same letter are not significantly different.

*3.3.2. Adults:* The results presented in Table (6) indicate significant differences in toxin concentrations among the adults of the studied fly species in relation to detecting poisoning-related homicides. The averages show that the adult blue flies achieved the highest significant concentration above the standard holding time concentration, reaching 8.797 µL/gram, followed closely by the average of flesh fly adults, which was 8.759 µL/gram.

In terms of individual concentrations, the adult blue flies and flesh flies excelled, providing four averages with concentrations exceeding the standard holding time concentration for each species, while the average concentrations of adult house flies were all below the standard holding time concentration. These results may indicate the ability of adult blue flies and flesh flies to retain traces of the toxin for a longer duration, as well as their sensitivity to the toxin and their ability to detect it effectively.

Table (6): Results of toxin concentration in the adults of the studied fly species at different intervals.

| Fly species | holding time/ minute | 15 /day | 18 /day | 21 /day | 25 /day | 27 /day | Means of species |
|---|---|---|---|---|---|---|---|
| *M. domestica* | 8.683 | 7.308 h | 7.196 i | 7.012 j | 6.625 k | 6.444 l | 6.917 c |
| *C. vicina* | 8.683 | 8.787 d | 8.743 e | 8.909 a | 8.671 g | 8.683 g | 8.759 b |
| *L. sericata* | 8.683 | 8.673 g | 8.729 f | 8.875 b | 8.872 b | 8.836 c | 8.797 a |

Means with the same letter are not significantly different.

Forensic entomology plays an increasingly important role in criminal investigations, providing valuable evidence in homicide cases through the analysis of fly larvae and adults. In a study by Costa *et al.* (2013), concentrations of lead (Pb), barium (Ba), and antimony (Sb), considered indicators of gunshot residues (GSR), were determined in the larvae of decaying flies from the family Calliphoridae. The concentrations in the fly larvae ranged from 6.28 to 1.78 micrograms/gram for Pb, 1.49 to 2.94 micrograms/gram for Ba, and 0.50 micrograms/gram and <LD for Sb, respectively.

LaGoo *et al.* (2010) evaluated the detection of gunshot residues (GSR) (9 mm Glock) in larvae of *Phaenicia sericata* collected during summer and winter using ICP-MS, obtaining LOD values ranging from 0.017 to 0.106 nanograms/mL, and LOQ values ranging from 0.10 to 1.0 nanograms/mL for lead (Pb), barium (Ba), and antimony (Sb). Motta *et al.* (2015) reported LOD values ranging from 0.15 to 4.79 micrograms/L and LOQ values from 0.50 to 15.97 micrograms/L when analyzing decaying larvae of *Crysomya albiceps*. Duarte estimated the shooting distance through the quantity of gunshot residues using larvae from the Calliphoridae family after a gunshot (9 mm Glock). In this study, the LOD values ranged from 0.01 to 0.05 micrograms/L using ICP-MS, with no LOQ values reported. The LOD and LOQ values obtained in this study are significantly lower than previous reports, indicating greater sensitivity of this method (Duarte, 2015).

Goff *et al.* (1989) demonstrated that toxic substances accumulated in fly larvae can positively or negatively influence fluctuations in length and weight, as well as the typical duration of their life cycles. Thus, these studies contribute to standardizing methodologies and data for estimating the postmortem interval (PMI), ensuring they meet the Daubert criteria for the admissibility of scientific evidence in court. A wide range of drugs and toxic substances has been detected in the larvae of various species, including primarily amitriptyline, propoxyphene, acetaminophen (Wilson *et al.*, 1993), steroids (Musvasva *et al.*, 2001), trazodone, trimipramine, and temazepam

(Sadler *et al*., 1995), methylphenidate (Bushby *et al*., 2012), heroin (Al-Qahtni *et al*., 2021), alcohol (Al-Khalifa *et al*., 2021), in addition to aluminum phosphide and diazinon (Cavalcante *et al*., 2023), etc.

Furthermore, when larvae actively feed on poisoned carcasses, foreign substances such as drugs and other toxins present in the tissues are absorbed into their metabolic systems. These substances can accumulate within the larvae's integument during growth and may be retained within the hardened pupa during pupation. Therefore, the pupa serves as a crucial source for toxic samples when skeletal remains are recovered. Additionally, many terrestrial arthropods are attracted to the odors emitted from decaying bodies. It is also important to note that toxic substances can significantly prolong the pre-appearance interval (PAI) for insects and delay oviposition (McIntyre *et al*., 2024). Investigating the effects of the pre-appearance interval (PAI) and oviposition on decay rates, microbial succession, and the production of volatile organic compounds can illuminate the role of toxins as drivers of postmortem changes. By integrating these factors, such as toxins and environmental influences, we can achieve a more comprehensive understanding of the complex ecological processes occurring during decomposition (Li *et al*., 2024).

## CONCLUSION

Each fly species demonstrates different efficiencies in detecting the crime-related substances used in this study, which may be linked to the life cycle of the adults or their capacity to absorb chemical substances. Based on these results, the blue fly may be the most sensitive to gunpowder, tramadol, and toxins. Additionally, the passage of time may affect the concentrations of these substances in the larvae and adults of the flies, highlighting the need for immediate analyses after collecting samples from the crime scene. Further analyses and experiments should be conducted to confirm these findings and determine the mechanisms employed by the studied fly species in detecting these chemicals.

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