



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies

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ARTICLE INFO

Article history:

Received 15 October 2020

Revised 17 November 2020

Accepted 10 December 2020

Available online 17 December 2020

Keywords:

Calliphoridae
Entomotoxicology
Dimethoate
And carrion flies

ABSTRACT

Forensic entomotoxicology has grown to impact judicial systems in developed countries. Where the use of insects and maggots as samples in death investigations as an alternative technique, especially following degradation or loss of the conventionally used samples. Carrion flies feed on dead bodies and may ingest toxic substances found in the dead body, especially when the body was poisoned before death. The knowledge of how the chemicals interact with the insect following ingestion is crucial to forensic entomotoxicologists. The study investigated the impact of dimethoate on the life cycle of four species of Calliphoridae flies, namely *Chrysomya megacephala*, *Chrysomya saffranae*, *Chrysomya rufifacies* and *Chrysomya indiana*. Various concentrations of dimethoate (1 ppm, 2 ppm, 3 ppm and 4 ppm) were utilized in the study. The rate of development of the carrion flies showed a negative correlation with the concentration of the chemical. This paper glares at the impact of the chemicals may pose to the insects, and how analysis of such impacts can guide forensic investigations of poisoning and help the investigators to solve the crime puzzle.

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1. Introduction

Forensic entomotoxicology is becoming popular in death investigations. The use of insects in forensic analysis of death is an alternative means of excluding or including poisoning even upon loss or degradation of convectional toxicological specimens. Various studies have demonstrated successful detecting, identifying, and quantification of chemicals from insects such as the Calliphoridae flies.

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Peer review under responsibility of King Saud University.



It is paramount to know the significance of insects in poisoning investigations as toxicological samples or evidence in criminal investigation (Chophi et al., 2019). Understanding the key methods of collecting, preserving, and analyzing insects' evidence is crucial in forensic science.

Maggots and insects can ingest drugs and other chemical substances found in the decaying corpse as they feed on it. The drug can be followed in the maggots and provide identity and concentration. Investigators have targeted the principal source of food-dependent upon by the larvae; the skeletal muscle (Pounder, 1991). Puparial analysis also needs to be considered to boost the admissibility of the evidence in the court of law. It is also paramount to evaluate the effects of the toxicological components to the insects and the maggots. Such can provide clues on what to expect as one follows the insects and maggots' chemical components. This study explores the effects of dimethoate on the developmental rate of the insects.

Carrion flies (Calliphoridae), found at crime scenes and dead bodies, have been used in forensic investigations to determine minimum post mortem interval, toxic substances, and whether

<https://doi.org/10.1016/j.sjbs.2020.12.022>

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the relocation of the body has occurred. Carrion flies can be used in toxicological analyses of forensic cases. Therefore, it is of key importance to determine the insects' precise morphology and the effects of suicidal chemicals on the insects. Knowledge of the effect of various toxicants on the carrion flies is paramount in forensic entomology. The toxicological analysis of the insects in crime scene investigations may guide the establishment of the cause of death or establish links that can determine the exact cause of death. The evidence held by insects in a crime scene is not prone to malicious destruction.

Dimethoate is an organophosphate used as pesticide. It is a common cause of poisoning in an accident, suicidal, and homicidal cases (Stephenson et al., 2006). It is vital to understand how the chemical interacts with the flies' systems commonly found in crime scenes (carrion flies). The presence of toxins such as dimethoate in the carrion can alter the developmental cycle of insects. Evidencing such alterations in blowflies at a crime scene may indicate dimethoate poisoning.

2. Materials and methods

2.1. Sample collection sites

Carrion flies (Calliphoridae) were collected from different places in Maharashtra state- India (Aurangabad, Jalgaon, Mumbai, Nandurbar, and Namded). The flies were collected from animal cadavers like street dogs, decaying meat and frish liver; the collection of larvae and adults was done following the standard method (Cooper and Cooper, 2013) with some modifications (Abd-Algalil et al., 2017). Forceps were used to pick the larvae, while sweep nets were used to collect the adults. The prepupae were putted in 500 ml beaker contain dry soil. The dry soil offers a suitable environment for pupation.

2.2. Samples identification

The collected samples were brought to the laboratory; larvae were reared separately in cages covered with muslin cloths. After the collection, pure cultures were prepared by collecting the eggs from each females and reared separately. For the morphological identification different stages of the collected species were dissected using fine needles under the stereo-zoom microscope (ERMA Optical works, Tokyo, No. 44883). The small dissected parts were mounted under the light microscope (Magnus Trinocular Microscope MLX-DX, Olympus -India PVT. LTD. No. 4B525145). The eggs were stained with potassium hydroxide (Sukontason et al., 2006) and observed under the light microscope. Morphological identification was done based on features and keys outlined (Abd-Algalil and Zambare, 2017; Sukontason et al., 2010).

2.3. Experimental design

Dimethoate is a universally known organophosphate insecticide (Stephenson et al., 2006). It is a contact insecticide. The organophosphate is associated with poisoning cases through accidental exposure, homicide, and suicide due to its availability in diverse agricultural use areas. TAFGOR Dimethoate 30% of equivalent concentration (EC) was used in the study. One milligram (1 ml) concentration of dimethoate was prepared by diluting 3.33 ml of 30% dimethoate with distilled water to prepare the stock solution. Different concentrations of the chemical were prepared, Fifty grams of sheep liver were chopped and mixed with 0.5 ml, 0.1 ml, 0.15 ml, and 0.2 ml dimethoate solutions to yield 1, 2, 3, and 4 ppm respectively. A sample of 50gms chopped liver was preserved as a control in the study. Sixty maggots within their first

instar were delivered into the prepared sample of the liver (Richards et al., 2013). The time taken for the maggots to undergo complete cycle for each concentration of dimethoate was investigated and recorded for each concentration at same condation of humidity and temperature for each species.

2.4. Statistical analysis

Using the GraphPad Software Inc. California, USA. (GraphPad Prism 8) Correlation analysis between the PMI and the dimethoate concentrations was carried out at a significance level of 0.05.

3. Results

3.1. Estimating minimum PMI

The duration of different stages of insect's development directly correlates to the Post Mortem Interval (PMI). Investigation of the growth durations of the different life cycle stages of the carrion flies (*Chrysomya megacephala*, *Chrysomya saffranaea*, *Chrysomya rufifacies* and *Chrysomya indiana*) was done to provide the minimum PMI as shown in the tables below.

3.2. Effect of dimethoate on *C. megacephala*

Dimethoate drugs undoubtedly influenced the life cycle duration and the different developmental stages. The life cycle duration varied depending on the concentration of the insecticide in the treated samples. The duration of the life cycle in the *C. megacephala* directly correlated with the concentration of dimethoate applied in different concentration. The samples treated with 1 ppm, 2 ppm, 3 ppm and 4 ppm took 310 h, 331 h, 355 h, and 373 h respectively (Table 1) while, the control took 286 h to complete their life cycle.

3.3. Effect of dimethoate on *C. saffranaea*

The control culture of *C. saffranaea* took 253 h for a complete life cycle. The treated insect's developmental stages lasted for 279 h, 301 h, 324 h, and 340 h with respect to the four different concentrations of the dimethoate (1 ppm, 2 ppm, 3ppm and 4 ppm) respectively (Table 2).

3.4. Effect of dimethoate on *C. indiana*

The duration of the life cycle of *C. Indiana* increased with an increase of dimethoate concentrations in ppm. The control culture lapsed for 246 h while the dimethoate treated cultures lapsed for 274 h, 291 h, 304 h, and 322 h, respective to the insecticide's rising in ppm concentrations (Table 3).

3.5. Effect of dimethoate on *C. rufifacies*

The total period from the first instar to adult fly took 267 h. But the duration of life cycle increased with an increase in the concentration of the dimethoate; 287 h, 301 h, 316 h, and 332 h respective to the increasing of the ppm concentrations (Table 4). The insects' live cycle was significantly shown the variation between the treated samples and the control in the same condition of temperature and relative humidity (Table 5).

The statistical analysis shown that the increase in dimethoate concentration was strongly associated with increases in PMI. Pearson's correlation coefficients ranged from (0.994–0.999) for all larvae, the PMI values for all the sampled blowflies were plotted against the concentrations of dimethoate (Figs. 1 and 2).

Table 1
Effect of dimethoate on life cycle duration of *Chrysomya megacephala*.

Duration of development from the eggs to adult	The total duration of development (PMI) <i>Chrysomya megacephala</i>	Temperature °C			Humidity %		
		Max	Min	Average	Max	Min	Average
Control	286 ± 2.10	27.1	23.7	25.4	62	47	54
Treated maggots	1 ppm 2 ppm 3 ppm 4 ppm	310 ± 1.22 331 ± 1.25 355 ± 2.20 373 ± 1.29					

± Standard deviation for five values.

Table 2
Effect of dimethoate on life cycle duration of *Chrysomya saffranae*.

Duration of development from the eggs to adult	Total duration of development (PMI) <i>Chrysomya saffranae</i>	Temperature °C			Humidity %		
		Max	Min	Average	Max	Min	Average
Control	253 ± 1.25	26.5	22.7	24.6	47	37	42
Treated maggots	1 ppm 2 ppm 3 ppm 4 ppm	279 ± 2.25 301 ± 2.12 324 ± 1.34 340 ± 1.67					

± Standard deviation for five values.

Table 3
Effect of dimethoate on life cycle duration of *Chrysomya indiana*.

Duration of development from the eggs to adult	Total duration of development (PMI) <i>Chrysomya indiana</i>	Temperature °C			Humidity %		
		Max	Min	Average	Max	Min	Average
Control	246 ± 1.50	26.6	24.3	25.5	44	34	39
Treated maggots	1 ppm 2 ppm 3 ppm 4 ppm	274 ± 2.11 291 ± 1.21 304 ± 1.15 322 ± 2.65					

± Standard deviation for five values.

Table 4
Effect of dimethoate on life cycle duration of *Chrysomya rufifacies*.

Duration of development from the eggs to adult	Total duration of development (PMI) <i>Chrysomya rufifacies</i>	Temperature °C			Humidity %		
		Max	Min	Average	Max	Min	Average
Control	267 ± 2.11	28.7	25.3	27.0	73	54	64
Treated maggots	1 ppm 2 ppm 3 ppm 4 ppm	287 ± 1.34 301 ± 1.45 316 ± 2.55 332 ± 1.85					

± Standard deviation for five values.

Table 5
Effect of dimethoate on life cycle duration of forensic importance Calliphoridae species.

Duration of development from eggs to adult		Total duration of development (PMI)			
		<i>Chrysomya megacephala</i>	<i>Chrysomya saffranae</i>	<i>Chrysomya rufifacies</i>	<i>Chrysomya indiana</i>
Control		286 ± 2.10	253 ± 1.25	267 ± 2.11	246 ± 1.50
Treated maggots	1 ppm	310 ± 1.22	279 ± 2.25	287 ± 1.34	274 ± 2.11
	2 ppm	331 ± 1.25	301 ± 2.12	301 ± 1.45	291 ± 1.21
	3 ppm	355 ± 2.20	324 ± 1.34	316 ± 2.55	304 ± 1.15
	4 ppm	373 ± 1.29	340 ± 1.67	332 ± 1.85	322 ± 2.65
Temperature °C	Max	27.1	26.5	28.7	26.6
	Min	23.7	22.7	25.3	24.3
	Average	25.4	24.6	27.0	25.5
Humidity %	Max	62	47	73	44
	Min	47	37	54	34
	Average	54	42	64	39

± Standard deviation for five values.

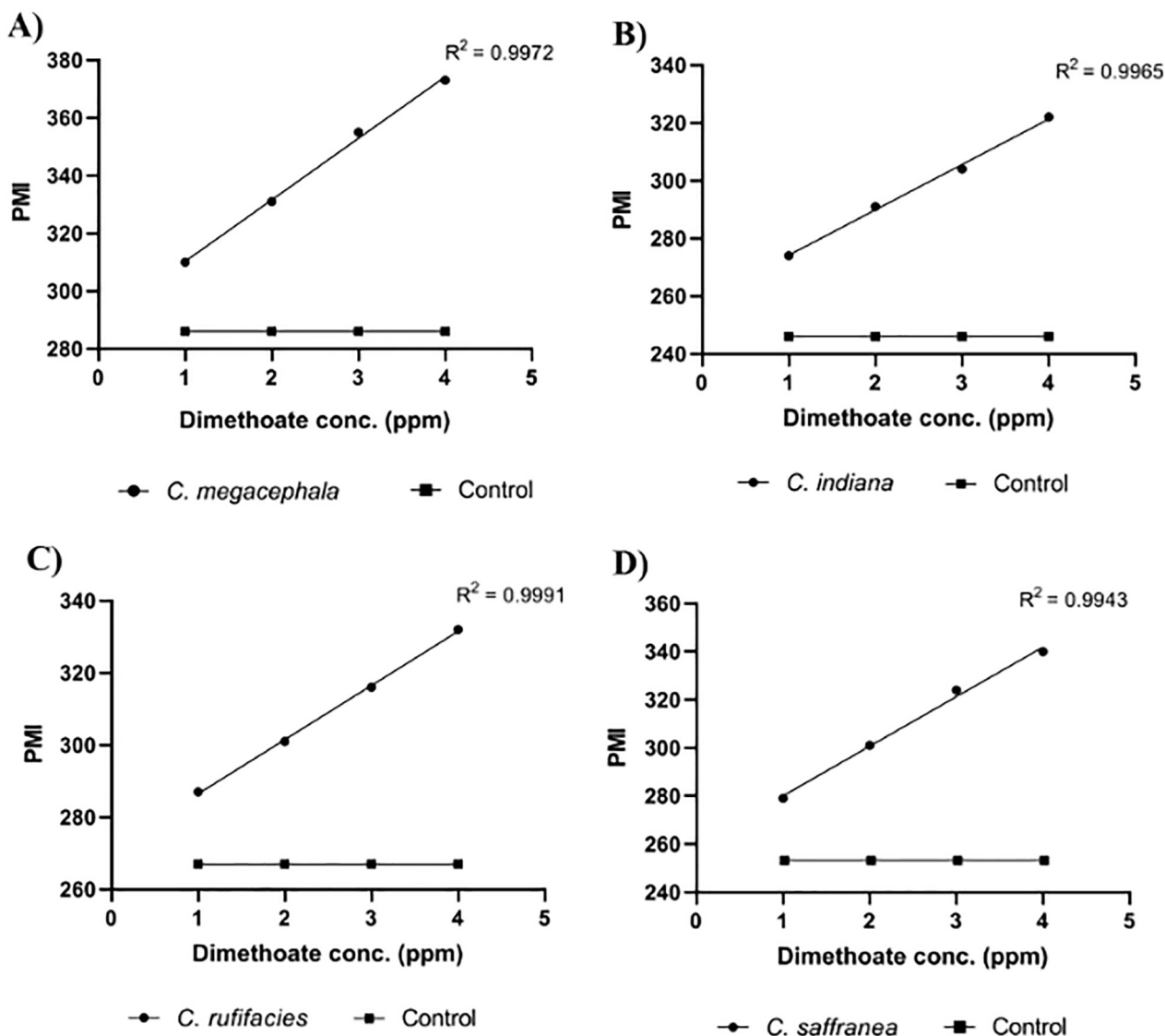


Fig. 1. Graphs A, B, C, and D represent the graphs of PMI against the concentrations of dimethoate for *C. megacephala*, *C. Indiana*, *C. rufifacies* and *C. saffrana*, respectively.

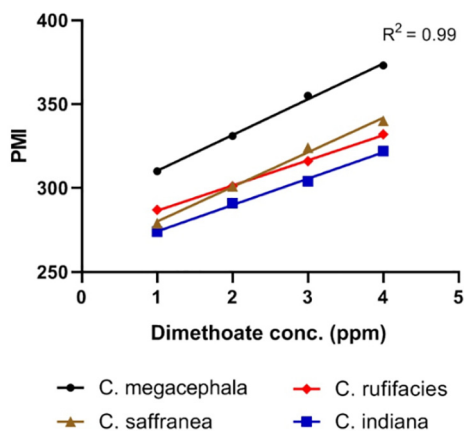


Fig. 2. A composite graph of the PMIs of the four Carrion flies against the concentrations of dimethoate.

4. Discussion

Dimethoate causes a delay in the development of carrion flies. The duration of the life cycle in all the species used in the study increased with an increase of dimethoate concentration. Dimethoate lengthens the feeding, post-feeding stages, and pupal stages of development of blowflies. The control culture illustrated the normal development of blowflies which provided a contrast to the test cultures. The results showed significant variations between the control and the treated larvae under the same environmental conditions corresponding to the results obtained from the study of the effects of malathion organophosphate on the growth rate of *C. megacephala* (Liu et al., 2009). When the larvae were fed on muscle and liver tissue treated with the organophosphate, their growth rates were retarded proportionally to the concentration of the chemicals.

The results also corresponded to (Yan-Wei et al., 2010) a study on the effect of malathion on the insect succession which pre-

sented a conceivable influence of the chemical on the developmental time of larval stages of insects and the decomposition rate. Such results were worth to conclude that the variations in the developmental rate of insect larvae were enough to change the PMI estimate. The study results concur with another study done on the effect of endosulfan on some carrion fly (Mali, 2011). Such has also been reported by Pawar (2011) in a study of how endosulfan affects development of *C. megacephala*.

The growth rate of calliphoridae larvae have been similarly affected by household products and contamination by hydrochloric acid, gasoline and various insecticides. Low concentrations decreases the survival rate while high concentrations have proved to be lethal. Some chemicals such as perfume and bleach barely alters the growth rate (Aubernon et al., 2015a; Aubernon et al., 2015b) and 2015a) or size of adult flies. Numerous studies have also indicated that various drugs and other toxic compounds can alter the developmental rate of maggot and interfere with estimations of postmortem intervals (Bourel et al., 1999; Bourel et al., 2001; Goff and Lord, 2001; Goff et al., 1992). The cause and manner of death can be forensically determined by investigating the chemicals isolated in tissues of the feeding stages of carrion flies. Unnormally reduced body size of adult carrion flies can establish links for further investigations of poisoning in severely decomposing human remains.

5. Conclusion

A delay in the growth and development of carrion flies found at a crime scene may be evidence of poisoning with chemicals such as dimethoate. Such evidence may establish links to follow while determining the cause of death. Carrion flies feed on cadavers and can ingest poisons that might have probably caused death to the body. Analysis of the duration of the life cycle can aid in estimating postmortem interval, which can, in turn, guide the establishment of the time of death. Therefore, I recommend the findings of the study to be considered in every death investigation at the crime scene to aid in ruling in or out poisoning as a cause of death.

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.

Acknowledgments

This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the fast-track Research Funding Program.

References

- Abd-Algalil, F.M., Zambare, S., Khan, L., Mali, K., 2017. Effect of seasonal temperature variations on the life cycle duration of forensically important calliphorid fly, *Chrysomya saffrana* (Bigot, 1877). *J. Forensic Res.* 8 (364), 2.
- Abd-Algalil, F.M., Zambare, S.P., 2017. Molecular identification of forensically important blowflies (Diptera: Calliphoridae) with a record of a new species from Maharashtra India. *Structure* 1, 3–6.
- Aubernon, C., Charabidze, D., Devigne, C., Delannoy, Y., Gosset, D., 2015a. Experimental study of *Lucilia sericata* (Diptera Calliphoridae) larval development on rat cadavers: effects of climate and chemical contamination. *Forensic Sci. Int.* 253, 125–130.
- Aubernon, C., Devigne, C., Hedouin, V., Gosset, D., Charabidze, D., 2015b. In vitro effects of household products on Calliphoridae larvae development: implication for forensic entomology. *J. Forensic Sci.* 60, 226–232.
- 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). *J. Forensic Sci.* 44 (2), 354–358.
- 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, 127–131.
- Chophi, R., Sharma, S., Sharma, S., Singh, R., 2019. Forensic entomotoxicology: current concepts, trends and challenges. *J. Forensic Legal Med.* 67, 28–36.
- Cooper, J.E., Cooper, M.E., 2013. *Wildlife Forensic Investigation: Principles and Practice*. CRC Press.
- 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*, pp. 331–340.
- Goff, M.L., Brown, W.A., Omori, A., 1992. Preliminary observations of the effect of methamphetamine in decomposing tissues on the development rate of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect on the estimations of postmortem intervals. *J. Forensic Sci.* 37, 867–872.
- Liu, X., Shi, Y., Wang, H., Zhang, R., 2009. Determination of malathion levels and its effect on the development of *Chrysomya megacephala* (Fabricius) in South China. *Forensic Sci. Int.* 192 (1–3), 14–18.
- Mali, K.H., 2011. Study of insects of crime investigation importance from nandurbar.
- Pawar, H.M., 2011. Study on Calliphorids of Forensic Importance from Aurangabad Ph.D. thesis submitted to Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.
- Pounder, D.J., 1991. Forensic entomo-toxicology. *J. Forensic Sci. Soc.* 31 (4), 469–472.
- Richards, C.S., Rowlinson, C.C., Hall, M.J., 2013. Effects of storage temperature on the change in size of Calliphora vicina larvae during preservation in 80% ethanol. *Int. J. Legal Med.* 127, 231–241.
- Stephenson, G.R., Ferris, I.G., Holland, P.T., Nordberg, M., 2006. Glossary of terms relating to pesticides (IUPAC Recommendations 2006). *Pure Appl. Chem.* 78, 2075–2154.
- Sukontason, K., Bunchu, N., Chaiwong, T., Moophayak, K., Sukontason, K.L., 2010. Forensically important flesh fly species in Thailand: morphology and developmental rate. *Parasitology Res.* 106, 1055–1064.
- Sukontason, K.L., Kanchai, C., Piangjai, S., Boonsriwong, W., Bunchu, N., Sripakdee, D., Chaiwong, T., Kuntalue, B., Siriwananarungsee, S., Sukontason, K., 2006. Morphological observation of puparia of *Chrysomya nigripes* (Diptera: Calliphoridae) from human corpse. *Forensic Sci. Int.* 161, 15–19.
- Yan-Wei, S., Xiao-Shan, L., Hai-Yang, W., Run-Jie, Z., 2010. Effects of malathion on the insect succession and the development of *Chrysomya megacephala* (Diptera: Calliphoridae) in the field and implications for estimating postmortem interval. *Am. J. Forensic Med. Pathol.* 31, 46–51.