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# Effects of insecticide dimethoate on the developmental rate of forensic importance sarcophagid flies

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ABSTRACT

The impact of forensic entomology on the judicial systems within the twenty-first century has earned its great considerations in matters of death investigations. Such is highly elaborate in developed countries. Malicious destruction of evidence is prominent in cases of homicides. Insect evidence is one of the tiny evidence that is hardly lost. Cadaveric insects feed on the dead bodies in succession manner. First witnesses arrive within minutes to the injured dead bodies, or within 24 h in the absence of the wounds. They ingest chemicals found in the cadvers. The chemical substances have diverse effects on the developmental rate and the life cycle duration of these insects. The effects alter the Post Mortem Interval (PMI) estimation.

The study focused on experimental investigations of the impact of dimethoate pose to sarcophagidae flies. The interaction of the chemicals with the tissues of the insect is phenomenal. Three species of sarcophagidae flies were used in the study *Sarcophaga peregrine, Sarcophaga dux,* and *Sarcophaga ruficornis*. Effects of various concentrations of dimethoate on such species were monitored under controlled conditions of humidity and temperature. The rate of development in the larval, prepupal, and pupal stages was investigated. It negatively correlated with the concentrations of dimethoate. The results were plausible enough to insure the dimethoate can alter the PMI determination, concurring with various studies in the science arena. Such investigations can establish useful links in following a crime. The study exploited a key phenomenon in medico-legal investigations.



## 1. Introduction

Forensic entomology entails the use of arthropods such as insects to aid in medico-legal investigations through uncovering circumstances of special interest. Entomotoxicolgy involves both qualitative and quantitative analysis of toxic compounds present in intoxicated tissues (Hall and Smith, 1993; Introna et al., 2001; Gosselin et al., 2011). These compounds can be found in insects that feed on the carrion. The cadaveric fauna comprise of flesh feeding arthropods that are the first witnesses of the crime (Voss et al., 2008). They include the Diptera such as Sarchophagidae family and Calliphoridae. They arrive at the crime scene within minutes following the discharge of blood and other body fluids. The second group of forensic importance cadaveric flies are the ones that feed on the bloat stage of decomposition (Rodriguez and Bass, 1983; Wolff et al., 2001) Thirdly, feeders of decaying matter, the Coleoptera, arrive following the post bloat and advanced decay stages of putrefaction of the dead body.

The occurrence of the first wave of arthropods as a witness of the crime depends on the climate, insect fauna in the geographical

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location covered or uncovered body, outdoor or indoor.. etc. Also the presence of body discharge such as blood (Campobasso and Introna, 2001). In the absence of the discharge, their arrival may be delayed to about twenty-four hours (Anderson, 1995). Presence of poisons in the tissues of the cadaver can be determined and quantified based on toxicological analysis of the cadaveric insect fauna, especially when the poison was administered before death (A Galal et al., 2009). It is paramount for a forensic entomologist to understand how the chemicals interact with the bodies of arthropods following ingestion. Understanding the effects of these chemicals pose to such arthropod witnesses is key in entomotoxicological analysis of death (Oliva, 2001). This branch of science is becoming popular and vital in death investigations. Malicious destruction of evidence in murder cases is elaborate in modern societies (Kyerematen et al., 2012) but the arthropod evidence is not easily lost. They ingest the poisons as they feed on these cadavers, the toxins interact with various tissues in the bodies of the insects. Recently ingested chemicals can be followed through gut content analysis (Sukontason et al., 2010). Non-recent ingestions can be investigated by analyzing the morphology, and size of the insects' body based on the knowledge of the effects of various chemical compounds on the insect (Centeno et al., 2002; Arnaldos et al., 2005) The chemicals can portray varied impacts on the cadaveric insects depending on the species and stages of development exposed to the chemicals. It is novel for forensic entomologists to understand the vital issues and methods employed in collection, preservation, and analysis of insect evidence (Goff et al., 1988).

Organophosphates affect both the feeding and non-feeding stages of the sarchophagidae flies. The effects are diverse and hence aid in determining the PMI (post mortem interval) or the time-lapse since the occurrence of death (VanLaerhoven, 2008; Abd Al Galil et al., 2020). Analysis of the first arthropod witnesses and other cadaveric fauna can provide an accurate estimation of the PMI. Analysis of the larval and puparial stages of the insects can boost the admissibility of the insect evidence by criminal investigators or the jury in the court (Grassberger and Frank, 2004; Segura et al., 2009). Entomological method of the PMI estimation provides the most superior and statistically reliable values of PMI (Marchenko, 1988) compared to autolysis, rigor mortis, and hypostasis which are based on classical postmortem alterations in soft tissues (Campobasso et al., 2001). The cadaveric fauna feeds in a successional manner based on the state or degree of composition (El-Kady et al., 1994). This research focused on the effect of dimethoate (organophosphate) on the developmental rate of the different life cycle stages of the first colonizers; the Sarchophagidae flies.

## 2. Material and methods

### 2.1. Sample collection

The first insects to colonize cadavers of street dogs and other animals cadavers (goat, and sheep) in Maharashtra state (Aurangabad, Mumbai, and Jalgaon) in India were collected. The adults, larvae and pupal sarchophagidae collection were based on the standard methods outlined by (Cooper and Cooper, 2013). The method was slightly modified based on (Abd-Algalil et al., 2017; Abd-Algalil, 2020). Adults were captured using sweep nets. The larvae were picked using pairs of forceps. The collected adults, pupae and larvae were reared in the laboratory separately. Adults were reared in the adult rearing cage, and the larvae were reared in larval rearing boxes, while the pupae were kept in container 500 ml beakers with dry soil. Adult and larvae fed on fresh liver of sheep daily and the adult were provided with 10 ml mixture of honey with water in the proportion of (10: 100 ml) respectively. The collected samples were identified morphologically after preparing the pure culture and the pure stalk were maintained for the further analysis (Abd-Algalil et al., 2017; Abd-Algalil et al, 2020).

### 2.2. Sample identification

From the prepared pure cultures of the collected samples, different developmental stages of Sarchophagidae flies were dissected with the help of fine needles under the stereo-zoom microscope; the ERMA optical works, No 44,883 Tokyo. The dissections were evaluated microscopically using Magnus Trinocular Microscope MLX-DX, Olympus PVT limited, NO 4B525145 India (Abd-Algalil et al., 2020). The dissected samples were morphologically identified based on key identification marks and keys postulated by (Sukontason et al., 2010). The immature stages were observed under the light microscope following similar methods of the clearing technique (Sukontason et al., 2004).

### 2.3. Expermintal design

#### 2.3.1. Treatment with dimethoate

The study used Dimethoate, 30% EC of the (TATA TAFGOR Company). Dimethoate is an organophosphatic known as contact insecticide capable of killing insects upon contact (Stephenson et al., 2006). Dimethoate is cheaply available in diverse agricultural applications and has a great association to the poisoning of both homicide and suicide or accidental exposure. Stalk solution of dimethoate was prepared by diluting the 3.33 ml of 30% solution in 1000 ml of distilled water to yield a one-milligram concentration. From the stalk solution, varied concentrations of 1 ppm, 2 ppm, 3 ppm and 4 ppm were prepared with 50gm of fresh minced liver. Four treated samples prepared with varying mentioned concentrations of dimethoate and the fifth sample was kept as control for each experiment of each species, the control contained only 50 g of fresh minced liver (Abd-Algalil, 2020; Richards et al., 2013). About 60 maggots, in their first instar of development, were introduced into the control and the test samples. The duration of the complete life cycle of the inoculated maggots was investigated in the control and the treated samples with insecticide. The results were recorded against temperature and humidity for all the concentrations and control.

#### 2.3.2. Postmortem interval (PMI) estimation

The developmental durations of different life cycle stages of the experimental samples were estimated as the basis of PMI (Sharma et al., 2015). These live cycle durations were correlate positively with the PMI.

### 2.4. Statistical analysis

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

## 3. Results

Dimethoate showed a great influence on growth (life cycle) at varying developmental stages. Its effects on the various species of Sarchophagidae are outlined in this study. Table 4 and Figs. 1 and 2, presents the data on the general effect of the insecticide on the development stages and the total life cycle duration of the three species of Sarchophagidae under study.

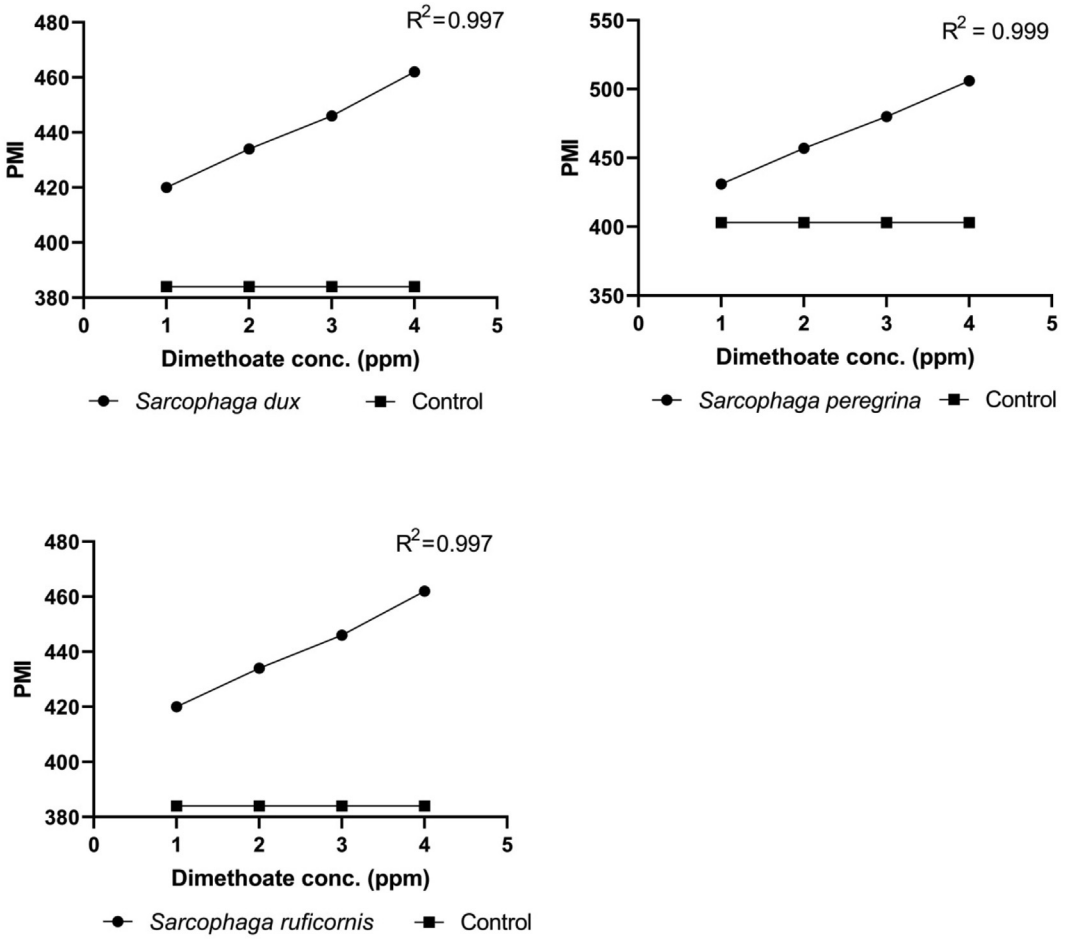


**Fig. 1.** Graphical representation of the effects of dimethoate on the three species of Sarcophagidae flies. PMI values were plotted against the concentration of dimethoate. The horizontal graph shows control cultures.

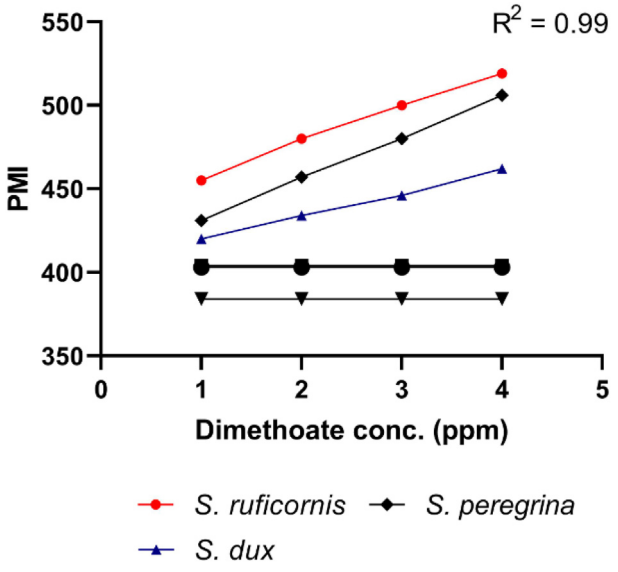


**Fig. 2.** The effects of dimethoate on the post mortem interval based on three Sarchophagidae species.

### 3.1. Effect of dimethoate on S. ruficornis

The organophosphate's effect on *Sarcophaga ruficornis* varied in intensity depending on the concentration of the dimethoate. The life cycle of untreated culture lapsed for 404 h. The life cycle of treated cultures lapsed for 455hrs, 480hrs, 500hrs, and 519hrs with respect to the increasing concentrations of 1 ppm, 2 ppm, 3 ppm and the 4 ppm respectively. The life cycle durations of the different species ware negatively correlated with an increase in the concentration of dimethoate. The average temperature and humidity were 25℃ and 54% respectively. (Table 1 and Fig. 1)

### 3.2. Effects of dimethoate on S. Dux

Dimethoate showed a non-negligible impact on the life cycle duration of the of *S. dux.* The control culture took 384 h while in the treatments, the duration varied based on the concentrations of the insecticide. The 1 ppm, 2 ppm, 3 ppm and 4 ppm cultures took 420, 434, 446 and 462 h respectively. It showed negative correlation with an increase in dimethoate concentration. The average temperature was 26.1℃ and the average humidity was 55% (Table 2 and Fig. 1).

**Table 1**
Effect of dimethoate on life cycle duration of *Sarcophaga ruficornis.*

| **Life cycle duration of *Sarcophaga ruficornis*f rom the 1st instar larvae to adult (PMI)** | | | **Temperature °C** | | | **Humidity %** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Max** | **Min** | **Average** | **Max** | **Min** | **Average** |
| **Control** | | 404 ± 2.15 | 26.5 | 23.4 | 25.0 | 60 | 48 | 54 |
| **Treated maggots** | 1 ppm | 455 ± 2.20 | | | | | | |
| | 2 ppm | 480 ± 1.50 | | | | | | |
| | 3 ppm | 500 ± 1.22 | | | | | | |
| | 4 ppm | 519 ± 1.78 | | | | | | |

± SD for five values.

**Table 2**
Effect of dimethoate on life cycle duration of *Sarcophaga dux.*

| **Life cycle duration of *Sarcophaga dux* from the 1st instar larvae to adult (PMI)** | | | **Temperature °C** | | | **Humidity %** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Max** | **Min** | **Average** | **Max** | **Min** | **Average** |
| **Control** | | 384 ± 1.25 | 27.7 | 24.4 | 26.1 | 63 | 47 | 55 |
| **Treated maggots** | 1 ppm | 420 ± 1.50 | | | | | | |
| | 2 ppm | 434 ± 2.20 | | | | | | |
| | 3 ppm | 446 ± 1.15 | | | | | | |
| | 4 ppm | 462 ± 2.30 | | | | | | |

± SD for five values.

### 3.3. Effects of dimethoate insecticide on S. peregrina

The life cycle duration of *S. peregrine* was greatly affected by the dimethoate insecticide. The control culture completed the full cycle in 403 h. The life cycles of the treated cultures ware complete in 431 h, 457 h, 480 h, and 506 h with respect to the ascending concentrations of dimethoate. Negative correlation between the life cycle duration with increasing concentrations of the dimethoate in the treated cultures, it is influenced on PMI estimation. The recorded average temperature and humidity were 25.4℃ and 55% respectively. (Table 3 and Fig. 1)

The study showed that the life cycle duration of the examined insect species of Sarcophagidae ware varied significantly, this clear variation between the treated samples and the control shoed in (Table 4) under the same condition of temperature and relative humidity.

Statistical analysis of the recorded data shown that the increase in dimethoate concentration was strongly associated with increases in PMI. Which means the dimetoate delay the life cycle duration in the treated samples compared to the control. The Pearson's correlation coefficients ranged from (0.994 – 0.999) for all larvae and the PMI values for all the species were plotted against the concentrations of dimethoate (Fig. 2).

**Table 3**
Effect of dimethoate on life cycle duration of *Sarcophaga peregrina.*

| **Life cycle duration of *Sarcophaga peregrine* from the 1st instar larvae to adult (PMI)** | | | **Temperature °C** | | | **Humidity %** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Max** | **Min** | **Average** | **Max** | **Min** | **Average** |
| **Control** | | 403 ± 2.15 | 27.1 | 23.6 | 25.4 | 64 | 47 | 55 |
| **Treated maggots** | 1 ppm | 431 ± 2.50 | | | | | | |
| | 2 ppm | 457 ± 1.20 | | | | | | |
| | 3 ppm | 480 ± 2.30 | | | | | | |
| | 4 ppm | 506 ± 1.50 | | | | | | |

± SD for five values.

**Table 4**
Effect of dimethoate on life cycle duration of forensic importance Sarcophgidae species.

| **Duration of development from eggs to adult** | | **The total duration of development (PMI)** | | |
|---|---|---|---|---|
| | | ***Sarcophaga ruficornis*** | ***Sarcophaga dux*** | ***Sarcophaga peregrina*** |
| Control | | 404 ± 2.15 | 384 ± 1.25 | 403 ± 2.15 |
| **Treated maggots** | 1 ppm | 455 ± 2.20 | 420 ± 1.50 | 431 ± 2.50 |
| | 2 ppm | 480 ± 1.50 | 434 ± 2.20 | 457 ± 1.20 |
| | 3 ppm | 500 ± 1.22 | 446 ± 1.15 | 480 ± 2.30 |
| | 4 ppm | 519 ± 1.78 | 462 ± 2.30 | 506 ± 1.50 |
| **Temperature °C** | Max | 26.5 | 27.7 | 28.7 |
| | Min | 23.4 | 24.4 | 25.3 |
| | Average | 25.0 | 26.1 | 27.0 |
| **Humidity %** | Max | 60 | 63 | 64 |
| | Min | 48 | 47 | 47 |
| | Average | 54 | 55 | 55 |

## 4. Discussion

Dimethoate has a profound effect on the development and morphology of sarcophagid flies. It is a cholinesterase inhibiting compound that delays the larval, pupal and prepupal stages of development. Increase in concentration of dimethoate is directly proportional to the duration taken in all the stages of development investigated. Both Sarcophargidae's feeding and post-feeding stages of development are lengthened by dimethoate. The normal duration of development was depicted by the control cultures, which provided a sharp contrast to the cultures under treatment (Yones et al., 2010). The variations in duration of development of among the test cultures and the control were significant even under similar conditions of humidity and pressure (Bourel et al., 2004). The PMI estimate is also affected by the insecticides. The values increase as concentration increases.

The results were in line with (Liu et al., 2009), where they investigated the effects of malathion on Calliphoridae's rate of development. The growth rates of insects are retarded by insecticides in proportion to the respective concentrations. Correspondence among the results to those of (Yan-Wei et al., 2010) where they reported profound impact of malathion on a succession of insects on the cadavers. The results showed no conflict with a study done on the effect of endosulfan insecticide on *L. cuprina* (Mali, 2011). The convergence of these studies is significant enough to conclude how insecticides and other organophosphatic compounds affect the development and morphology of insects. The results show thatthe life cycle duration and PMI were negatively affected by toxic compounds such as the insecticides (Abd El-bar and Sawaby, 2011; Attia, 2002). The impact intensifies with an increase in the concentration of the chemicals. Such results to alterations in the PMI estimates.

The understanding of the effects of succession pattern applied by insects on cadavers is ideal to a forensic entomologist. They aid in the establishment of the PMI estimates or the time-lapse since the contested death occurred(Varatharajan and Sen, 2000) Analysis of such insect evidence can guide the establishment of the cause and manner of death especially in incidences of poisoning and drug abuse (GOFF and LORD, 2009). It can be accomplished through analysis of the toxins found in tissues of insects collected from the crime scene.

## 5. Conclusion

Developmental delay is the ultimate result following ingestion of toxins by insects. Insects collected from a crime scene may suggest poisoning, especially if they show retardation in growth to a significant degree. Sarcophagidae flies are the first group of insects to colonize a dead body. The flies ingest poisons that might have possibly killed the victim. These chemicals can be analyzed to rule out poisoning in death investigations. They provide an estimation of PMI important in establishing time of death. Toxicological analysis of the insects found in crime scenes should be emphasized in 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.

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