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An entomotoxicological study on the influence of house hold toxins on the colonization of carrion by Sarcophaga haemorrhoidalis

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Abstract: *the aim of this study was to examinethe effects of house hold toxins such as Allout insect killer and Maxo insect repellent in different environmental condition on the colonization of carrion by invertebrates. Allout and Maxo were used because of its common house hold use as an insecticide and its ubiquitous use in society. The investigations aimed to examine the possible effects of these toxins in field-based testing for the estimation of Post-mortem interval. The field-based testing was done using bovine liver with a solution of Allout and Maxo injected into the tissues. The samples were protected from feeding and removal by vertebrate scavengers. It was found that the presence of Allout and Maxo significantly affected the time taken for Diptera to colonise the biological tissue. The development/completion of life cycle stages of flesh flies in the sample treated with Allout was found to be faster by 2 days in open area and by 1 day in closed area when compared to the controls in the respective conditions. The development of I instar larvae in the sample containing Maxo was found to be delayed by a period of 4 days in open area and by 2 days in closed area. Therefore, environmental changes and presence of toxins in the tissues play an important role in the life cycle of forensically important flies and hence all these factors must be considered in the Post Mortem Interval determination.*
Keywords: *Diptera, Entomotoxicology, Insecticides, PMI.*

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I. Introduction

Forensic entomology plays an important role in the pursuit of justice. The pathologist can usually provide a reasonably accurate determination of the time since death in the first 72 hours after death.Historically, this has been based upon the condition of the body and such parameters as: lividity, rigor mortis, post-mortem cooling, changes in the chemical constituents of body, autolysis of tissue, and decomposition due to bacterial activity in the body. The pathologist depends on rigor mortis, livor mortis, and core temperature of the corpse to derive acceptable estimates within 24 to 36 hours of death. Insects offer a much longer time scale measured in hours, days, weeks, or even years. [1]

When death occurs, cells begin to die and enzymes begin to digest the cells inside out in a process called autolysis. The body starts decomposing. Gastrointestinal tract bacteria start to degrade the soft tissues producing liquids and gases like hydrogen sulphide, carbon dioxide, methane, ammonia, Sulphur dioxide and hydrogen. The volatile molecules called apeneumones escaping from the decomposing body attract insects. The volatile molecules released during each stage can modify the insect behaviour. [2]It was found in 1950 that putrative Sulphur-based compounds were responsible for attracting the flies to the decomposing carcass, but egg laying or oviposition of the flies were facilitated by ammonium-rich compounds present on the carrion. [3]

Several factors can affect how a carcass is decomposed by invertebrates. A research suggests that if carrion is to be considered an ecosystem, then chemicals present in the body prior to death can be considered a form of pollution.[4]The study of such toxins and their interactions with insects is called entomotoxicology.[5] Entomotoxicology is the analysis of toxins on arthropods that feed on the carrion. Careful analysis of the insect population found on a decomposing body, combined with knowledge of insect biology, ecology, and local environmental conditions can often provide valuable forensic insights.These can include the estimation of time since death, movement of the remains after death, indication of ante mortem injuries, and the presence of drugs or toxins. This "cadaver pollution" can take the form of many different toxins, which in turn can have many effects on the carrion ecosystem. Entomotoxicology is generally split into two distinct branches, the first looks at the effect of chemicals on the development of insects feeding upon carrion, the second looks at the detection of toxins within insects feeding upon corpses.

II. Material And Methods

This study was conducted in Bangalore (urban area), India from 20th February 2019 (22 sampling days), and a secondary sampling period ran from 20th March 2019 (20 sampling days). The materials used included; Larval forceps, plastic containers and plastics specimen cups, hygro-thermometer for measuring temperature and humidity, preserving solution (formalin), disposable gloves and Log book.

Rearing of blowflies

Each experiment used 100g of fresh bovine liver as bait, as this has proven successful in trapping flies in previous studies. The Bait was placed in a trap to prevent scavenging by other animals.Three samples, one injected with 5 ml Allout, one injected with 5 ml Maxo and one without any poison was set as control and was placed in an open dry environment. The same experimental setup was repeated and the samples were placed in a closed room environment. The temperature and humidity were recorded daily. The development stage of all the samples was recorded on a timely basis.

Collection and preservation of specimens

Samples of insects of all stages were collected from different areas of the liver, insects were often found to congregate in locations resembling wounds and natural orifices.

Specimens were found as:-

- Eggs (in egg masses usually)
- Larvae or maggots
- Pupae and/or empty pupal cases
- Adults.

Eggs - They are very tiny and are usually laid in clumps or masses, and are usually found in a wound or natural orifice. They were collected with help of a paint brush dipped in water or with forceps and preserved in formalin.

Maggots - A range of sizes were collected. Maggots were found to be crawling on or near the remains and were present in maggot masses. Large maggots are usually older and are most important, but smaller maggots were also collected as they may belong to a different species. The samples of maggots from different areas of the liver and the surrounding area were collected and kept in separate specimen vials. The third instar larvae leave the food source to find a suitable area to pupate. When collected, the samples were divided into two – half for preservation and half kept alive in a clean vial in order to allow them to transform into their pupae.

Pupae and empty pupal cases - Pupae like dry and secure areas away from the wet food source in which to pupate. They range from 10-20 mm, and are oval in shape. They are dark brown when completely tanned. An empty pupal case is very similar but is open at one end, where the adult fly has emerged. The empty pupal cases were preserved without the preserving solution for the purpose of identifying the species.

Adult flies - They are used for indicating which species of insect are likely to develop from the pupa. The emerged adult flies are kept without food and then preserved as such.

Sampling

Table 1: Different types of sample prepared for studying the entomological cycle

S. No	Conditions given			
	Open Area	**Sample Coding**	**Closed Area**	**Sample Coding**
1	Control	Sample 1	Control	Sample 4
2	Treated with All Out	Sample 2	Treated with All Out	Sample 5
3	Treated with Maxo	Sample 3	Treated with Maxo	Sample 6

III. Observations And Result

In the present study the life cycle stages of Sarcophagahaemorrhoidaliswere recorded which consisted of larvae (i.e. 1st instar, 2nd instar and 3rd instar), pupa and adult. The duration of life cycle stages changed as per the temperature variations and the presence of different toxins present in the samples.

1. Sample Condition: Open Area

Sample prepared Date/Time: 20 February 2019, 10:00 am

Inoculation Date/Time: 20 February 2019, 1:00 pm

The post-mortem interval of all the bait samples used here was 22 days. The time duration of different life cycle stages of flies in the control (sample 1) was completed in 22 days from larviposition to emergence of adult fly at the average temperature ranging from 18.3°C - 41°C and average humidity ranging from 20% – 66%. However when the sample in the same temperature and humidity range were treated with Allout (sample 2), the life spans

of the fly was completed in 19 days and when treated with Maxo (sample 3), it took 20 days for the life cycle of the fly to be completed.

1.1 Sample 1: Control

Table 2 shows that in the control (sample 1) the 1st instar larval development took 24 hours. The 1stinstar larvae took 1day to moult into 2nd instar larvae. The 3rdinstar larvae development started 3 days after eggs were deposited and continued up to 7th day after which the pupa stage was developed. The adult emerged out from the pupae after 14 days of development. Therefore the developmental duration of flies was in accordance with the post-mortem interval.

Table 2: Time duration of different stages of Sarcophaga haemorrhoidalis in the control (sample 1)

Life cycle stages		**Duration**
Eggs		24 hours
Larva	1st Instar	2nd day
	2nd Instar	3rd day
	3rd Instar	4th day
Pupa		5-7 days
Fly		8-22 days
Total duration		22 days

1.2 Sample 2: Treated with All Out

In the sample treated with Allout (sample 2) the 1st instar larval development took 3 days from the time the bait was placed. The 1stinstar larvae took 1day to moult into 2ndinstar larvae. The 3rdinstar larvae development started 3 days after eggs were deposited and continued up to 8th day after which the pupa stage was developed. The adult emerged out from the pupae after 11 days of development. A delay of 1 day was observed in estimating PMI.

Table 3: Time duration of different stages of Sarcophaga haemorrhoidalis in the sample treated with All Out (sample 2)

Life cycle stages		**Duration**
Eggs		2nd day
Larva	1st Instar	3rd day
	2nd Instar	4th day
	3rd Instar	5th day
Pupa		6-8 days
Fly		9-20 days
Total duration		20 days

1.3 Sample 3: Treated with Maxo

In the sample treated with Maxo (sample 3) the 1st instar larval development was delayed for a period of 4 days after which the 1st instar larvae took 2 day to moult into 2nd instar larvae. The 3rd instar larvae development started 5 days after eggs depositing and continued up to 10th day after which the pupa stage was developed. The adult emerged out from the pupae after 11 days of development. A delay of 2 day was observed in estimating PMI.

Table 4: Time duration of different stages of Sarcophaga haemorrhoidalis in the sample treated with Maxo(sample 3)

Life cycle stages		**Duration**
Eggs		3rd day
Larva	1st Instar	4th day
	2nd Instar	6th day
	3rd Instar	7th day
Pupa		8-10 days
Fly		11-22 days
Total duration		22 days

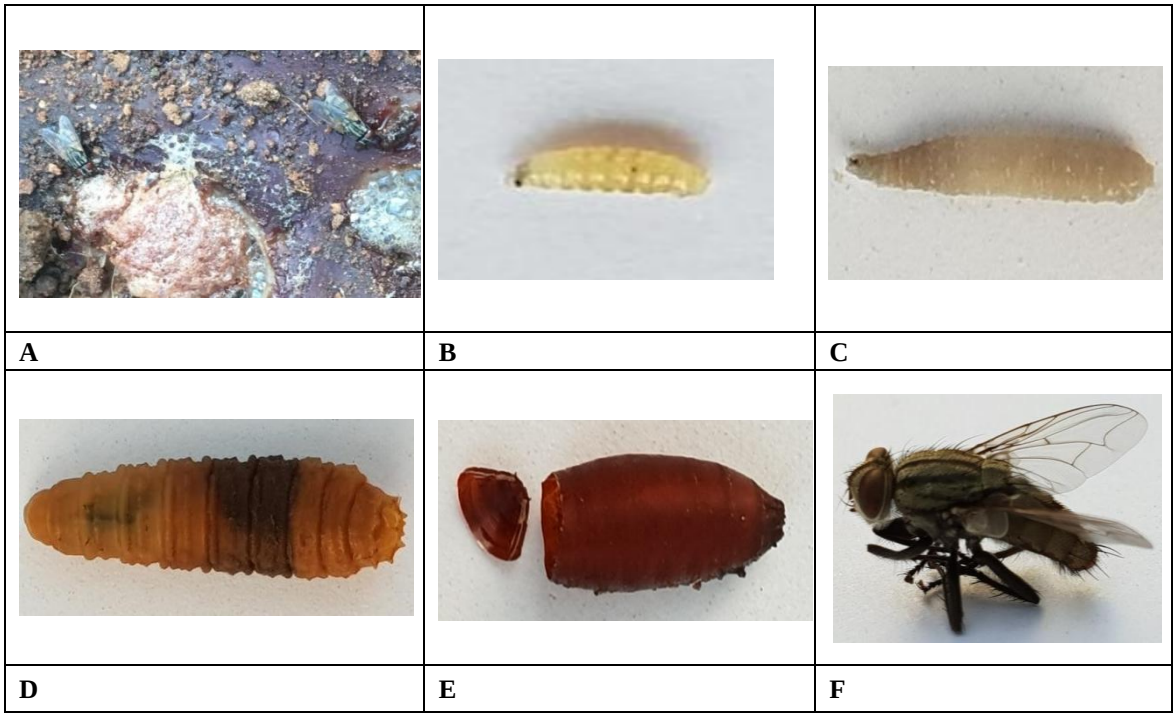

Figure 1:- Life cycle stages of Sarcophaga haemorrhoidalis in open area.(A)Bait to attract adult flies; (B) 1st instar larvae; (C) 2nd instar larvae; (D) 3rdinstar; (E)empty pupal case; (F) Adult fly

2. Sample Condition: Closed Area
Sample prepared Date/Time: 20 March 2019, 9:30 am
Inoculation Date/Time: 20 March 2019, 1:00 pm

The post-mortem interval of all the liver samples used here was 20 days. In closed environment the time duration of different life cycle stages of flies in the control (sample 4) was completed in 20 days from larviposition to emergence of adult fly at the average temperature ranging from 27-30.3ºC and average humidity ranging from 29– 38%. However when the sample in the same temperature and humidity range were treated with Allout (sample 5), the life spans of the fly was completed in 19 days and when treated with Maxo (sample 6), it took 20 days for the life cycle of the fly to be completed.

2.1 Sample 4: Control

In the control (sample 4) the 1st instar larval development took 24 hours. The 1st instar larvae took 1day to moult into 2nd instar larvae. The 3rd instar larvae development started 3 days after eggs were deposited and continued up to 7th day after which the pupa stage was developed. The adult emerged out from the pupae after 12 days of development. The post-mortem interval estimation from the life cycle stages of flies was accurate.

Table 5: Time duration of different stages of Sarcophaga haemorrhoidalis in the control sample. (Sample 4)

Life cycle stages		**Duration**
Eggs		24 hours
Larva	1st Instar	2nd day
	2nd Instar	3rd day
	3rd Instar	4th day
Pupa		5-7 days
Fly		8-20 days
Total duration		20 days

2.2 Sample 5: Treated with All Out

In the sample treated with Allout (sample 5) the 1st instar larval development took 22 hours. The 1st instar larvae took 1day to moult into 2nd instar larvae. The 3rd instar larvae development started 4 days after eggs were deposited and continued up to 6th day after which the pupa stage was developed. The adult emerged out from the pupae after 9 days of development. A delay of 1 day was seen in estimating PMI.

Table 6: Time duration of different stages of Sarcophaga haemorrhoidalis in the sample treated with All Out (sample 5)

Life cycle stages		**Duration**
Eggs		22 hours
Larva	1st Instar	2nd day
	2nd Instar	3rd day
	3rd Instar	4th day
Pupa		6-9 days
Fly		10-19 days
Total duration		19 days

2.3 Sample 6: Treated with Maxo

In the sample treated with Maxo (sample 6) the 1st instar larval development was delayed for a period of 2 days after which the 1st instar larvae took 1 day to moult into 2nd instar larvae. The 3rd instar larvae development started 3 days after eggs were deposited and continued up to 8th day after which the pupa stage was developed. The adult emerged out from the pupae after 11 days of development. The PMI was delayed by 1 day.

Table 6: Time duration of different stages of Sarcophaga haemorrhoidalis in the sample treated with Maxo (sample 6)

Life cycle stages		**Duration**
Eggs		24 hours
Larva	1st Instar	2nd day
	2nd Instar	3rd day
	3rd Instar	4th day
Pupa		5-8 days
Fly		9-20 days
Total duration		20 days

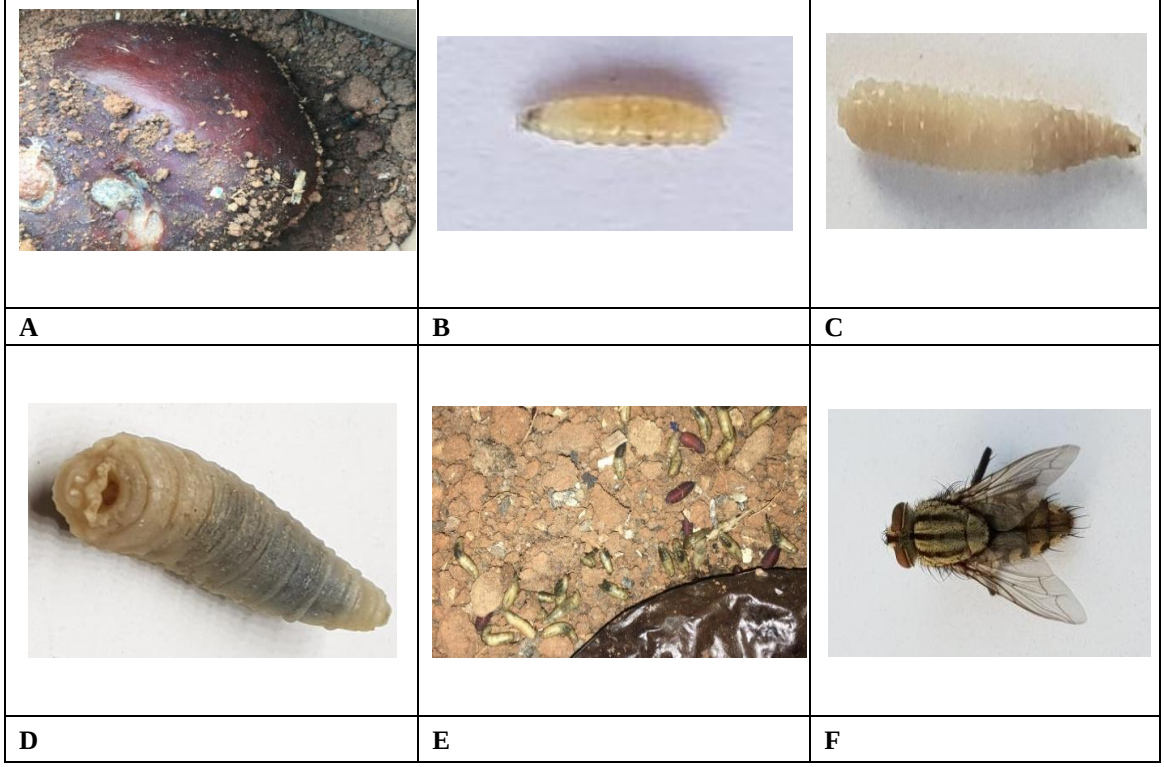

Figure 2:- Life cycle stages of Sarcophaga haemorrhoidalis in closed area.(A) Bait to attract adult flies; (B) 1st instar larvae; (C) 2nd instar larvae; (D) 3rdinstar; (E)Pupa formation; (F) Adult fly

IV. Conclusion

Entomotoxicology involving studies on toxins effects on growth phase of insect development are to be seriously taken in the forensic investigation of crime scenes in order to get an accurate estimate of time since death especially from the remains of decomposed bodies. When the effects of the toxins on the growth rates were observed, a clearly distinct change was seen in the growth pattern. Insecticides such as Allout and Maxo caused significant differences in maggots feeding on treated meat, compared to an untreated control in field conditions. The development of life cycle stages of flies in the sample treated with Allout was found to be faster by 2 days in open area and by 1 day in closed area when compared to the controls in the respective conditions. The development of I instar larvae in the sample containing Maxo was found to be delayed by a period of 4 days in open area and by 2 days in closed area. The number of larvae observed also showed significant differences with the maximum reproduction occurring with the control sample, followed by the Allout and Maxo showing the least number of larvae. The presence of these toxins in tissue remains can affect the development of maggots and therefore estimates of PMI. Hence all of this study's parameter shows that the variations in environmental conditions and toxin existence influence the growth and colonization of forensically significant flies.A similar study investigated the effects of drugs ethanol and cannabis on growth rates of the chrysomya rufifacies where the control sample took an average of 4 days to grow from 1st instar to pupae stages whereas the samples grown in the presence of ethanol and cannabis showed a much faster growth rates. [6] Delay of up to 29 hours can occur in PMI estimates with heroin containing tissues; similar results were reported for methamphetamine and amitriptyline. [7]

Arthropods prove to be valuable tools in the investigation.In addition to the known applications for estimating the post-mortem period, in the absence of body tissues and fluids normally sampled for such purposes, arthropods can also serve as reliable alternative specimens for toxicological analysis. While the data reviewed above concerning the potential effects of poisons on rates of insect development are limited in scope and no adverse impacts on case analyses have been reported to date, it is not unreasonable to assume that such substances, found in tissues fed upon by carrion insects, have the potential for altering developmental patterns. Entomotoxicology may prove to be another valuable tool in the forensic science arsenal.

References

[1]. Greenberg B. a. (2002). Entomology and the Law: Flies as Forensic Indicators. Cambridge University Press. New York
[2]. LeBlanc HN, Logan JG. Exploiting Insect Olfaction in Forensic Entomology. In: Amendt J, Goff ML, Campobasso CP, Grassberger M, editors. Current Concepts in Forensic Entomology. Netherlands: Springer; 2010. pp. 205–21.
[3]. Ashworth JR, Wall R. Responses of the sheep blowflies Lucilia sericata and Lxuprina to odour and the development ofsemi chemical baits. Med Vet Entomol. 1994; 8:303–9.
[4]. Chick, A. (2014). The Effect of Nicotine on Carrion Feeding Insects with Considerations for Use within Forensic Sciences. Nottinghamshire.
[5]. Introna and Francesco. "Entomotoxicology." Forensic Science International. 120 (2001): 42-47.
[6]. Verma, K. (2013). Forensic Entomology world: A new study on Chrysomya rufifacies from India. Journal of Entomology and Zoology Studies, 125-141.
[7]. Kapil Verma, R. P. (2013). Assessment of Post Mortem Interval, (PMI) from Forensic Entomotoxicological Studies of Larvae and Flies. Entomology, Ornithology & Herpetology.

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