Journal of Bioanalysis & Biomedicine

Verma, J Bioanal Biomed 2013, 5:1
DOI: 10.4172/1948-593X.1000074

Research Article Open Access

Effects of Codeine, Sodium Pentothal and Different Temperature Factors on the Growth Rate Development of Chrysomya rufifacies for the Forensic Entomotoxicological Purposes

Kapil Verma*

M.Sc Forensic Science, Amity Institute of Forensic Sciences (AIFS), B-Block, Lower Ground Floor, Amity University, Sec-125, Noida-201303, Uttar Pradesh, India

Abstract

In the above study the growth and colonization of blow flies of species *Chrysomya rufifacies* (Diptera: Calliphoridae) were studied under different environmental conditions at Noida, Uttar Pradesh, India. On the basis of result it was clearly seen that a changes in temperature and humidity bring about a significant changes in growth pattern of the larval stages. In the condition with the higher temperature larva developed quickly and matured into pupa when compared to the sample grown in cooler temperature. It was also noted that fly larva grew and mature faster when they were placed under humid conditions. This study investigated the effects of drugs ethanol and cannabis on growth rates of the blowfly. Where the control sample took an average of 4 days to grow from 1st instar to pupae stages, the samples grown in the presence of ethanol and cannabis showed a much faster growth rates. Therefore it can be concluded that both the studies that were put forward before the start of this study have been proven and that the differences in environmental conditions and presence of drugs affect the growth and colonization of blow flies. This study demonstrates again the necessity of considering the possible effects of drugs in tissues on insect growth rates when estimating the postmortem interval (PMI) using entomological techniques.

Keywords: Blow fly; Larval stages; Ethanol; Cannabis; Control sample; Instars; Pupae stages; Colonization; Effect of drugs, PMI; Entomological techniques

Introduction

Forensic Entomology is the use of insects and other arthropods in forensic investigation concerning decomposed bodies and it has become the "gold standard" for estimating time since death in many countries. In addition to estimating the post-mortem interval (PMI) insects that feed on carcasses may also represent a reliable specimen for toxicological analyses (Entomotoxicology) [1-3]. It is very useful for cases where the body has been long dead. Different Species of insects lay eggs on dead compost, Forensic entomologists done research on this kind of insects and their larval lifecycles finally they determine the body has been dead before three days or four days ago. After three days of investigation, insect evidence is most accurate in some method of determining duration time since death. Recently, I have also analyzed this kind of cases' in which duration time since death was only a few hours previous to discovery [4-6].

Two main ways of using insects to determine duration time since death, by using succession ally waves of insects, using maggot age and its development in three different methods as follows, The first method is used when the corpse has been dead for between a month up to a year or more, and the second method is used when death occurred less than a month prior to discovery [7-10].

Materials and Methods

Entomology kit; Insects net, Collecting vials, Larval forceps ,Wide mouth bottles, Plastic containers and plastics specimen cups, Thermometer for measuring tem, Chamber, Camera, Preserving solution, Disposable gloves, Dropper and pipettes, Shipping containers, Vermiculite, Ruler/tape, Log book [11-14].

1. These samples were collected randomly from the meat shops. Meat kept in open environment in Noida and was subjected for collection.
2. The sample flies collected were subjected for collection and rearing of flies.
3. These flies were identified as *Chrysomya rufifacies*.
4. 50 flies were used in this study, placed in 12 jars (4 each). These flies were allowed to rear under different environmental conditions and different drugs ethanol, and cannabis.
5. Vermiculite was filled in rearing chamber.
6. 12 jars placed to observe the colonization of the blow flies.
7. Meats were placed inside the jars treated with drugs.
8. 8 jars were placed in 4 different environmental conditions contained meat that had been treated with different drugs.
9. 4 flies were transferred into each jar.
10. Jars were placed under the different conditions;

- Cool temperature (Humid) 20-24°C
- Cool temperature (Dry) 18-22°C
- Room temperature (Humid) 26-30°C
- Room temperature (Dry) 24-28°C

***Corresponding author:** Kapil Verma, M.Sc Forensic Science, Amity Institute of Forensic Sciences (AIFS), B-Block, Lower Ground Floor, Amity University, Sec-125, Noida-201303, Uttar Pradesh, India, E-mail: forensic.kapilalert@gmail.com

Received January 10, 2013; **Accepted** February 25, 2013; **Published** February 28, 2013

Citation: Verma K (2013) Effects of Codeine, Sodium Pentothal and Different Temperature Factors on the Growth Rate Development of *Chrysomya rufifacies* for the Forensic Entomotoxicological Purposes. J Bioanal Biomed 5: 006-012. doi:10.4172/1948-593X.1000074

11. All the observation were noted /recorded day by day.
12. From the point of 1st appearance of larva, closely counts of larva/pupa were made time to time until all larvae had reached the pupa stage.

Results

Control sample

Condition: Room temperature (Dry): The jar containing adult blow flies were placed at room temperature on the 11th march. The eggs were observed to have been laid by the 14th march. On the 3rd day after incubation the 1st in star stage was observed. From which point counting was performed after 6 hour. By the 78th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 90 hours was of the pupa (Tables 1 and 2).

Control sample

Condition: Room temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 26th march. On the 4th day after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 78th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 84 hours was of the pupa (Tables 3 and 4).

Date of observation	Observation
11th March	4 flies placed in jars
12th	No activity
13th	1 fly dead
14th	2 fly dead, eggs laid
15th	1 fly dead,1st instar, (2 mm)
16th	2nd instar (9 mm)
17th	3rd instar (16 mm)
18th	pupae

Table 1: It shows observation day wise of the jar containing the flies placed for copulation.

Hours	No. of larvae/pupae
6	23
12	39
18	50
24	65
30	70
36	74
42	79
48	81
54	83
60	85
66	85
72	86
78	88 (larvae/pupae)
84	88 (larvae/pupae)
90	91 (pupae)

Table 2: Count of larvae taken every 6 hours after first appearance larvae (15th march).

Date of observation	Observation
26th March	4 flies placed in jars
27th	No activity, 1 adult fly dead
28th	2 fly dead, eggs laid
29th	1 fly dead, 1st instar (2 mm)
30th	2nd instar (7 mm)
31st	3rd instar (16 mm)
1st April	pupae

Table 3: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of larvae/pupae
6	32
12	43
18	56
24	66
30	72
36	78
42	82
48	86
54	89
60	91
66	94
72	95
78	96 (larvae/pupae)
84	95 (pupae)

Table 4: Count of larvae taken every 6 hours after first appearance larvae (29th march).

Date of observation	Observation
26th March	4 adult flies placed in jars
27th	No activity, 1 adult fly dead
28th	No activity, 2 adult fly dead
29th	No activity, 1 adult fly dead
30th	Eggs laid
31st	1st instar (2 mm)
1st April	2nd instar (7 mm)
2nd	2nd instar (13 mm)
3rd	3rd instar (17 mm)
4th	pupae

Table 5: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of larvae/pupae
6	14
12	26
18	35
24	43
30	49
36	56
42	63
48	66
54	68
60	71
66	72
72	73
78	71
84	71
90	68 (larvae/pupae)
96	67 (larvae/pupae)
102	65 (pupae)

Table 6: Count of larvae taken every 6 hours after first appearance larvae (31st march).

Date of observation	Observation
26th March	4 flies placed in jars,1 fly dead
27th	No activity, 2 fly dead
28th	No activity, 1 fly dead
29th	No activity,
30th	Eggs laid
31st	1st instar (3 mm)
1st April	2nd instar (9 mm)
2nd	2nd instar (14 mm)
3rd	3rd instar (17 mm)
4th	pupae

Table 7: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of larvae/pupae
6	19
12	32
18	47
24	53
30	61
36	65
42	70
48	74
54	76
60	77
66	78
72	78
78	77
84	77 (larvae/pupae)
90	76 (larvae/pupae)
96	74 (pupae)

Table 8: Count of larvae taken every 6 hours after first appearance larvae 31st March.

Control sample

Condition: Room temperature (Dry): The jar containing adult blow flies were placed at room temperature on the 26th march. The eggs were observed to have been laid by the 30th march. On the 6th day after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 90th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 102 hours was of the pupa (Tables 5 and 6).

Control sample

Condition: Room temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 26th march. On the 6th day after incubation the 1st instar stage of larvae were observed. The 1st instar stages of larvae were first observed on the 31th march. From which point counting was performed after 6 hour. By the 84th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 96 hours was of the pupa (Tables 7 and 8).

Ethanol treated sample

Condition: Room temperature (Dry): The jar containing adult blow flies were placed at room temperature on the 15th march. The eggs were observed to have been laid by the 17th march. On the 4th day (18th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 66th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 72 hours was of the pupa (Tables 9 and 10).

Ethanol treated sample

Condition: Room temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 26th march. The eggs were observed to have been laid by the 28th march. On the 5th day (30th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 54th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 66 hours (2nd April) was of the pupa (Tables 11 and 12).

Ethanol treated sample

Condition: Cool temperature (Dry): The jar containing adult blow

Date of observation	Observation
15th March	4 flies placed in jars
16th	no activity, 2 flies dead
17th	No activity, 2 flies dead, eggs laid
18th	1st instar, (2 mm)
19th	2nd instar, (7 mm)
20th	2nd instar, (11 mm)
21st	3rd instar, (16 mm)
22nd	pupae

Table 9: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	18
12	30
18	42
24	59
30	68
36	71
42	76
48	80
54	82
60	84
66	85
72	87
78	88 (larvae/ pupae)
84	89 (larvae/ pupae)
90	88 (larvae/ pupae)
96	88 (larvae/ pupae)
102	87 (larvae/ pupae)
108	87 (larvae/ pupae)
114	87 pupae

Table 10: Count of larvae taken every 6 hours after first appearance larvae 18th march.

Date of observation	Observation
26th March	4 flies placed in jars
27th	no activity, 1 fly dead
28th	3 flies dead, eggs laid
29th	No activity
30th	1st instar, (1 mm)
31st	1st instar, (4 mm)
1st April	2nd instar, (9 mm)
2nd	3rd instar, (13 mm)
3rd	3rd instar, (17 mm)
4th	pupae

Table 11: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	31
12	42
18	54
24	67
30	73
36	79
42	83
48	85
54	87
60	88
66	91
72	93 (larvae/ pupae)
78	95 (larvae/ pupae)
84	96 (larvae/ pupae)
90	96 (larvae/ pupae)
96	95 (larvae/ pupae)
102	95 (larvae/ pupae)
108	94 (pupae)

Table 12: Count of larvae taken every 6 hours after first appearance larvae 30th march.

Date of observation	Observation
26th March	4 flies placed in jar
27th	No activity, 2 fly dead
28th	No activity 2 flies dead
29th	Eggs laid
30th	No activity
31st	No activity
1st	1st instar, (3 mm)
2nd 3rd	2nd instar (8 mm) 2 instar (11 mm)
4th	3rd instar (16 mm)
5th	pupae

Table 13: Observation day wise of the jar containing the flies placed for copulation.

flies were placed at room temperature on the 26th march. On the 4th day (29th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 66th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 84 hours was of the pupa (Tables 13 and 14).

Ethanol treated sample

Condition: Cool temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 26th march. The eggs were observed to have been laid by the 29th march. On the 5th day (30th march) after incubation, the 1st in star stage of larvae were observed. From which point counting was performed after 6 hour. By the 54th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 66 hours was of the pupa (Tables 15 and 16).

Cannabis treated sample

Condition: Room temperature (Dry): The jar containing adult blow flies were placed at room temperature on the 15th march. The eggs were observed to have been laid by the 17th march. On the 4th day (18th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 72th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 78th hours was of the pupa (Tables 17 and 18).

Hours	No. of Larvae / pupae
6	4
12	13
18	23
24	32
30	40
36	46
42	54
48	58
54	62
60	63
66	64
72	66
78	67
84	67 (larvae/ pupae)
90	68 (larvae/ pupae)
96	67 (larvae/ pupae)
102	67 (larvae/ pupae)
108	66 (larvae/ pupae)
114	64 (larvae/ pupae)
120	63 pupae

Table 14: Count of larvae taken every 6 hours after first appearance larvae 1st april.

Date of observation	Observation
26th March	4 flies placed in jar
27th	No activity, 1fly dead
28th	Eggs Laid, 3 flies dead
29th	No activity
30th	1st in star, (2 mm)
31st	1st in star, (5 mm)
1st	2nd instar (9 mm)
2nd	2nd instar (11 mm)
3rd	3rd instar (15 mm)
4th	pupae

Table 15: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	5
12	18
18	27
24	38
30	45
36	55
42	62
48	64
54	66
60	68
66	72
72	74
78	75
84	74 (larvae/ pupae)
90	74 (larvae/ pupae)
96	73 (larvae/ pupae)
102	72 (larvae/ pupae)
108	72 (larvae/ pupae)
114	71 (larvae/ pupae)
120	71 (pupae)

Table 16: Count of larvae taken every 6 hours after first appearance larvae 30th march.

Cannabis treated sample

Condition: Room temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 26th march. The eggs were observed to have been laid by the 28th march. On the 4th day (29th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 60th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 72 hours was of the pupa (Tables 19 and 20).

Cannabis treated sample

Condition: Cool temperature (Dry): The jar containing adult blow flies were placed at room temperature on the 26th march. The eggs were observed to have been laid by the 29th march. On the 4th day (30th march) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 72th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 84 hours was of the pupa (Tables 21 and 22).

Cannabis treated sample

Condition: Cool temperature (Humid): The jar containing adult blow flies were placed at room temperature on the 2nd April. The eggs were observed to have been laid by the 5th April. On the 5th day (6th April) after incubation the 1st instar stage of larvae were observed. From which point counting was performed after 6 hour. By the 66th hour, the counting was made of a mixture of a larva and pupa and the final reading taken at 72 hours was of the pupa (Tables 23 and 24).

Date of observation	Observation
28th March	4 flies placed in jars, 1 fly dead
29th	NO activity,1 fly dead
30th	No activity, 2 flies dead, eggs laid
31st	No activity
1st April	1st instar, (2 mm)
2nd	2nd instar, (9 mm)
3rd	2nd instar (12 mm)
4th	3rd instar (16 mm)
5th	pupae

Table 17: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	13
12	26
18	38
24	47
30	55
36	63
42	65
48	66
54	69
60	71
66	73
72	76
78	77
84	77 (larvae/ pupae)
90	78 (larvae/ pupae)
96	78 (larvae/ pupae)
102	79 pupae

Table 18: Count of larvae taken every 6 hours after first appearance larvae 1st april.

Date of observation	Observation
28th March	4 flies placed in jars, 1 fly dead
29th	NO activity, 2 fly dead
30th	No activity, 1 fly dead,
31st	eggs laid, 1st instar, (2 mm)
1st April	2nd instar, (9 mm)
2nd	2nd instar, (13 mm)
3rd	3rd instar, (17 mm)
4th	Pupae

Table 19: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	24
12	38
18	49
24	57
30	63
36	68
42	74
48	76
54	79
60	80
66	83
72	85
78	84 (larvae/ pupae)
84	84 (larvae/ pupae)
90	83 pupae

Table 20: Count of larvae taken every 6 hours after first appearance larvae 31st march.

Date of observation	*Observation*
28th March	4 flies placed in jars, 2 flies dead
29th	no activity, 2 fly dead
30th	No activity
31st	eggs laid
1st April	1st instar, (2 mm)
2nd	2nd instar, (8 mm)
3rd	2nd instar, (12 mm)
4th	3rd instar (17 mm)
5th	pupae

Table 21: Observation day wise of the jar containing the flies placed for copulation.

Discussion and Conclusion

In the above study the growth and colonization of blow flies of species *Chrysomya rufifacies* were studied under different conditions. On the basis of result it was clearly seen that a changes in temperature and humidity bring about a significant changes in growth pattern of the larval stages.

In the condition with the higher temperature larva developed quickly and matured into pupa when compared to the sample grown in cooler temperature. It was also noted that fly larva grew and mature faster when they were placed under humid conditions [15-17].

When the effects of the toxins on the growth rates were observed, a clearly distinct change was seen in the growth pattern. Where the control sample took an average of 4 days to grow from 1st instar to pupae stages, the samples grown in the presence of ethanol and cannabis showed a much faster growth rates.

Hours	No. of Larvae/pupae
6	8
12	14
18	25
24	33
30	39
36	45
42	50
48	56
54	61
60	64
66	67
72	68
78	69
84	70
90	69 (larvae/ pupae)
96	68 (larvae/ pupae)
102	67 (larvae/ pupae)
108	66 pupae

Table 22: Count of larvae taken every 6 hrs after first appearance larvae 1th april.

Date of observation	Observation
28th March	4 flies placed in jars, 2 flies dead
29th	No activity, 2 adult fly dead
30th	No activity
31st	eggs laid
1st April	1st instar (2 mm)
2nd	2nd instar (8 mm)
3rd	3rd instar (17 mm)
4th	pupae

Table 23: Observation day wise of the jar containing the flies placed for copulation.

Hours	No. of Larvae/pupae
6	5
12	16
18	26
24	37
30	43
36	50
42	55
48	60
54	65
60	68
66	70
72	71
78	72 (larvae/ pupae)
84	73 (larvae/ pupae)
90	74 (larvae/ pupae)
96	74 (pupae)

Table 24: Count of larvae taken every 6 hours after first appearance larvae 1st april.

The number of larvae observed also showed significant differences with the maximum reproduction occurring with the control sample, followed by the cannabis and ethanol showing the least number of larvae [18-23].

Therefore it can be concluded that both the studies that were put forward before the start of this study have been proven and that the differences in environmental conditions and presence of drugs affect the growth and colonization of blow flies [24,25]. This study demonstrates again the necessity of considering the possible effects of drugs in tissues on insect growth rates when estimating the postmortem interval (PMI) using entomological techniques.

References

1. Byrd JH, Butler JF (1998) Effects of temperature on Chrysomya rufifacies (Diptera: Calliphoridae) development. J Med Entomol 34: 353-358.
2. Baumgartner DL (1986) The hairy maggot blow fly *Chrysomya rufifacies* (Macquart) confirmed in Arizona. Journal of Entomological Science 21: 130-132.
3. Byrd JH (1995) The effects of temperature on flies of forensic importance. M.S. thesis. University of Florida 197.
4. Baumgartner DL (1993) Review of Chrysomya rufifacies (Diptera: Calliphoridae). J Med Entomol 30: 338-352.
5. Catts EP (1992) Problems in estimating the postmortem interval in death investigations. Journal of Agricultural Entomology 9: 245-255.
6. Corry JEL (1978) Possible sources of ethanol ante- and post-mortem: Its relationship to the biochemistry and microbiology of decomposition. Journal of Applied Bacteriology 44: 1-56.
7. Commack JA, Nelder MP (2010) Cool-weather activity of the forensically important hairy maggot blow fly Chrysomya rufifacies (Macquart) (Diptera: Calliphoridae) on carrion in Upstate South Carolina, United States. Forensic Sci Int 195: 139-142.
8. Davies L, Radcliffe GG (1994) Development rates of some pre-adult stages in blowflies with reference to low temperature. Med Vet Entomol 8: 245-254.
9. Davis GL, Leffert RL, Rantanen NW (1972) Putrefactive ethanol sources in post-mortem tissues of conventional and germ free mice. Arch Pathol 94: 71-74.
10. Deonier CC (1940) Carcass temperatures and their relation to winter blowfly populations and activity in the southwest. Journal of Economic Entomology 33: 166-170.
11. Monthei DR (2009) Entomotoxicological and Thermal Factors Affecting the Development of Forensically Important Flies, Faculty of Virginia Polytechnic Institute, Blacksburg 1-112.
12. Figarola JLM, Skoda SR (1998) *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) in Nebraska. J Entomol Sci 33: 319-321.
13. Goodbrod JR, Goff ML (1990) Effects of larval population density on rates of development and interactions between two species of Chrysomya (Diptera: Calliphoridae) in laboratory culture. J Med Entomol 27: 338-343.
14. Goff ML, Lord WD (1994) Entomotoxicology: a new area for forensic investigation. Am J Forensic Med Pathol 15: 51-57.
15. Tabor KL, Fell RD, Brewster CC, Pelzer K, Behonick GS (2005) Effects of ante mortem ingestion of ethanol on insect successional patterns and development of Phormia Regina (Diptera: Calliphoridae). J Med Entomol 42: 481-489.
16. Kapil Verma, Reject Paul MP (2012) Assessment of Post Mortem Interval, PMI) from Forensic Entomotoxicological study on growth rates of larvae of flies 64.
17. Meek CL, Puskarich-May C, Carlton CE (1998) New state record for the hairy maggot blow fly Chrysomya rufifacies (Macquart) Southwest Entomol 23: 373-375.
18. Roy DN, Siddons LB (1939) the life history and bionomics of *Chrysomya rufifacies* Macq. (Order Diptera, Family Calliphoridae). Parasitology 31: 442-447.
19. Subramanian H, Mohan KR (1980) Biology of the blow flies Chrysomya megacephala, Chrysomya rufifacies, and Lucilia cuprina. Kerala Journal of Veterinary Science 11: 252-261.
20. Shiao SF, Yeh TC (2008) Larval competition of *Chrysomya megacephala and Chrysomya rufifacies* (Diptera: Calliphoridae): Behavior and ecological studies of two blow fly species of forensic significance. J Med Entomol 45: 785-799.
21. Sukontason K, Piangjai S, Siriwattanarungsee S, Sukontason KL (2008) Morphology and developmental rate of blowflies *Chrysomya*

megacephala and *Chrysomya rufifacies* in Thailand: application in forensic entomology. Parasitology Research 102: 1207-1216.

22. Van den Oever R (1976) A review of the literature as to the present possibilities and limitations in estimating the time of death. Med Sci Law 16: 269-276.

23. Van Laerhoven SL (2008) Blind validation of postmortem interval estimates using developmental rates of blow flies. Forensic Sci Int 180: 76-80.

24. Vargas J, Dickerson ED, Davis MP (2001) United States prescribing habits: what's going on? (Abstract). Support Care Cancer 9: 311.

25. Vinogradova EB, Marchenko MI (1984) The use of temperature parameters of fly growth in the medico legal practice. Sudebno-meditsinskaya Ekspertiza 27: 16-19.