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

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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

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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).

| 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 every 6 hours after first appearance larvae (15th march).

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 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 3 and 4).

| Date of observation | Observation |
|---------------------|-------------|
| 26th March          | 4 adult 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.
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).

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 flies were placed at cool 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, 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).
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 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 13 and 14).

**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 84th hours was of the pupa (Tables 13 and 15).

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

| 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 (larvae / pupae)  |

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

| 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                 | 2nd instar (8 mm)             |
| 3rd                 | 2nd instar (11 mm)            |
| 4th                 | 3rd instar (16 mm)            |
| 5th                 | pupae                         |

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

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

| Date of observation | Observation                   |
|---------------------|-------------------------------|
| 26th March          | 4 flies placed in jar         |
| 27th                | No activity, 1 fly 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 16: Count of larvae taken every 6 hours after first appearance larvae 30th march.**

| 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)           |
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 2nd April. 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 66th hour, counting was made of a mixture of a larva and pupa and the final reading taken at 84 hours was of the pupa (Tables 23 and 24).

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 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 21 and 22).

| 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 instar.

| 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 instar.

| 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.
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.

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