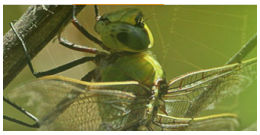

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Forensic Entomology world: A new study on *Chrysomya rufifacies* from India

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This study aims to determine the usefulness and applicability of forensic entomology in the Indian perspective, to develop an understanding of insect lifecycle, awareness of beneficial insect, understanding of forensic entomology and increase critical thinking application for student to further their interest in future career goals in entomology. Forensic Entomology is very useful for cases where the body has been long dead. After some days, insect evidence is often the most accurate and sometimes the only method of determining elapsed time since death. In the above study the life cycle of *Chrysomya rufifacies* were studied under different temperatures Cool temperature (Humid) 20-24^{0}C, Cool temperature (Dry) 18-22^{0}C, Room temperature (Humid) 26-30^{0}C and Room temperature (Dry) 24-28^{0}C and the effects of drugs on growth or colonization of *Chrysomya rufifacies* at Noida, Uttar Pradesh, India. In this study the species used *Chrysomya rufifacies* showed that its growth cycle is affected by fluctuation in temperatures and these factors can be considered as an estimate of the minimum time of death.

Keyword: Drugs, Colonization, Effect of Drugs, PMI, Entomotoxicological Techniques.

1. Introduction

Forensic entomology is the study of insects associated with a dead body and has been used and accepted in courts around the world. It is primarily used to determine time of death [1]. In addition to estimating the post-mortem interval (PMI) insects that feed on carcasses may also represent a reliable specimen for toxicological analyses (Entomotoxicology) [2-3]. Determining time of death is extremely important in a death investigation as it focuses the investigation into the correct time frame. Most forensic Entomological studies have concentrated on PMI studies, drugs related deaths. However, after some days, insect evidence is often the most accurate and sometimes the only method of determining elapsed time since death [4-5]. The method used is determined by the circumstances of each case [6-8].

Chrysomya rufifacies is also widely distributed throughout the world. It is more adapted to tropical conditions and is found throughout the year [9]. This species has been used as a forensic indicator by many scientists [10-12]. If we know how long it takes to reach the different stages in an insect's life, we can calculate the time since the egg was laid. This calculation of the age of the insects can be considered as an estimate of the minimum time of death. But even if the estimate of the insect age is correct, the death of the victim occurred before the eggs were laid.

Entomological knowledge can reveal the manner or location of death, but is most often used to estimate the time of death, or postmortem interval. Two time-dependent processes may be involved here. The first is the growth of insect larvae that feed upon the victim. Most of the carrion insects rarely deposit offspring on a live person, therefore the age of a larva provides a minimum time since death. The second is the succession of carrion-arthropod species found in the body, which has the potential of providing

both a minimum and maximum estimated post-mortem interval [13].

2. 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 [14-15].

1. The beef meats were collected randomly from the meat shops. In total 4kg of buffalo beef liver were subjected for collection and rearing of flies.
2. The beef liver kept in open environment in Noida and was subjected for collection of flies.
3. The flies collected using net from the open field Noida was introduced into the container kept in the laboratory
4. Different species of flies were collected from the exposed decomposed meat in the open environment in Noida.
5. The flies were used in this study were *Chrysomya rufifacies*
6. Vermiculite was filled in rearing chamber.
7. Beef liver were also placed inside the jars treated with drugs
8. Fly *Chrysomya rufifacies* were placed in jars. These flies were allowed to rear under different environmental conditions and different drugs Ethanol, and Cannabis.
9. Jars were placed in rearing chamber at 4 different environmental conditions.
10. Jars were placed under the different conditions;
    a. Room temperature (Dry) 24-28^{0}C
    b. Room temperature (Humid) 26-30 ^{0}C
    c. Cool temperature (Dry) 18-22^{0}C
    d. Cool temperature (Humid) 20-24 ^{0}C
11. All the observation was noted day by day.

From the point of 1st appearance of larva, closely counts of larva and pupa were made time to time until all larvae had reached the pupa stage

3. Results:

3.1 Control sample:

3.1.1 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 (Table 1 and Table 2).

Table 1: Show Observation Day Wise of the Jar Containing the Flies

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

Table 2: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae (15th march)

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)

3.2 Control sample:

3.2.1 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 (Table 3 and Table 4).

Table 3 shows observation day wise of the jar containing the flies

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

Table 4 shows count of larvae taken every 6 hours after first appearance larvae (29th march)

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)

3.3 Control sample:

3.3.1 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 (Table 5 and Table 6).

Table 5 shows observation day wise of the jar containing the flies

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

Table 6 Shows count of larvae taken every 6 hours after first appearance larvae (31st march)

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)

3.4 Control sample:

3.4.1 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 (Table 7 and Table 8).

Table 7: shows observation day wise of the jar containing the flies

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

Table 8: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae

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)

Control sample shows count of larvae taken every 6 hrs after 1st appearance larva below in Figure 1.

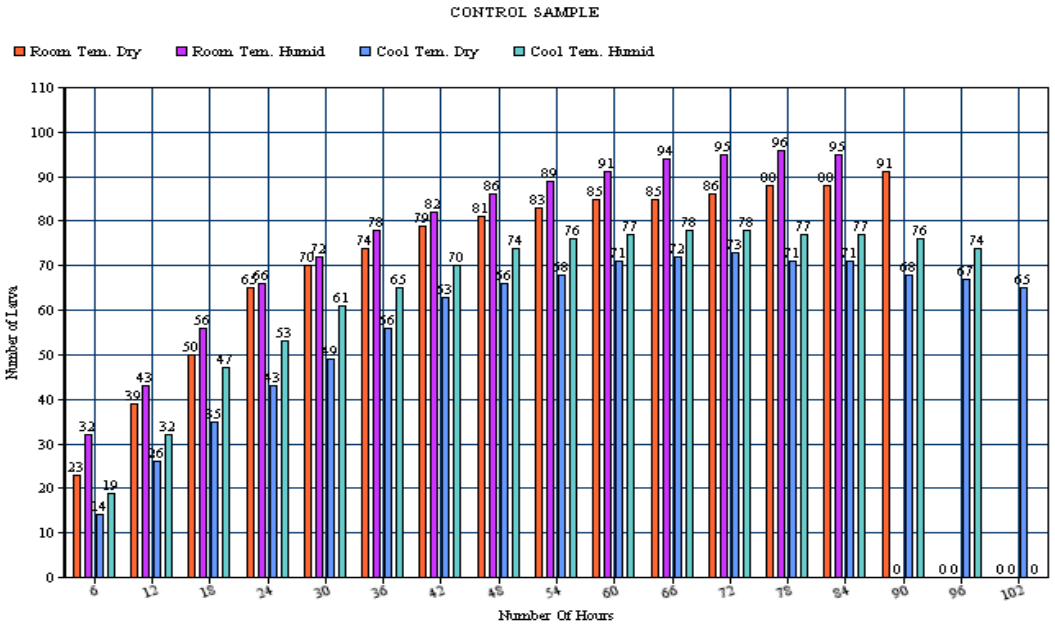

Fig 1: Control Sample Shows Count of Larvae Taken Every 6 hrs after 1st Appearance Larva

3.5 Ethanol Treated Sample

3.5.1 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 (Table 9 and Table 10).

Table 9: shows observation day wise of the jar containing the flies

Date of observation	Observation
26th March	4 flies placed in jars,
27th	No activity, 1 fly dead
28th	Eggs laid, 2 flies dead
29th	1st instar, (2mm), 1 adult fly dead
30th	2nd instar,(8mm)
31st	3rd instar,(17mm)
1st April	Pupae

Table 10: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 29th march

Hours	No. of larvae/pupae
6	19
12	32
18	45
24	58
30	65
36	73
42	78
48	85
54	87
60	88
66	86 (*larvae/ pupae)*
72	85 *pupae*

3.6 Ethanol Treated Sample

3.6.1 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 (Table 11 and Table 12).

Table 11: Shows Observation Day Wise of the Jar Containing The Flies

Date of observation	Observation
26th march	**4** adult flies placed in jars, 1adult fly dead
27*th*	**N**o activity,1 adult fly dead
28*th*	Eggs laid, 3adult flies dead
29*th*	No activity
30*th*	1st instar, (3mm)
31*st*	2nd instar, (8mm)
1*st*	3rd instar, (16mm)

2nd	**P***upae*

Table 12: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 30th march

Hours	No. of larvae/pupae
6	29
12	43
18	55
24	66
30	72
36	78
42	86
48	89
54	92(*larvae/ pupae)*
60	93(*larvae/ pupae)*
66	92 (*pupae)*

3.7 Ethanol Treated Sample

3.7.1 Condition: Cool temperature (Dry)

The jar containing adult blow 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 (Table 13 and Table 14).

Table 13: Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
26*th* March	**4** adult flies placed in jar, 1 fly dead
27*th*	**N**o activity, 1 fly dead
28*th*	**N**o activity, Eggs laid
29*th*	*2* flies dead, 1st instar, (1mm)
30*th*	2nd instar,(7mm)
31*st*	2nd instar,(12mm)
1*st*	**3***rd* instar,(16mm)
2nd	**P***upae*

Table 14: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 29th march

Hours	No. of larvae/pupae
6	**11**
12	**18**
18	**26**
24	**36**
30	**46**
36	**51**
42	**57**
48	**60**
54	**63**
60	**65**
66	**66(***larvae/ pupae)*
72	**69(***larvae/ pupae)*

78	**70**(*larvae/ pupae)*
84	**72** *pupae*

3.8 Ethanol Treated Sample

3.8.1 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 (Table 15 and Table 16).

Table 15 Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
26*th* March	**4** adult flies placed in jar, 1adult fly dead
27*th*	**N**o activity,1 adult fly dead
28*th*	Eggs laid, 2 *adult* flies dead
29*th*	**No activity**
30*th*	1st in star,(2mm)
31*st*	2nd in star,(8mm)
1*st*	**3***rd* **i***nstar*,**(16***mm***)**
2nd	*Pupae*

Table 16 Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 30th march

Hours	No. of larvae/pupae
6	**14**
12	**19**
18	**29**
24	**43**
30	**52**
36	**60**
42	**65**
48	**67**
54	**69**(*larvae/ pupae)*
60	**69**(*larvae/ pupae)*
66	**68**(*pupae)*

Sample treated with Ethanol shows count of larvae taken every 6 hrs after 1st appearance larva below in Figure 2.

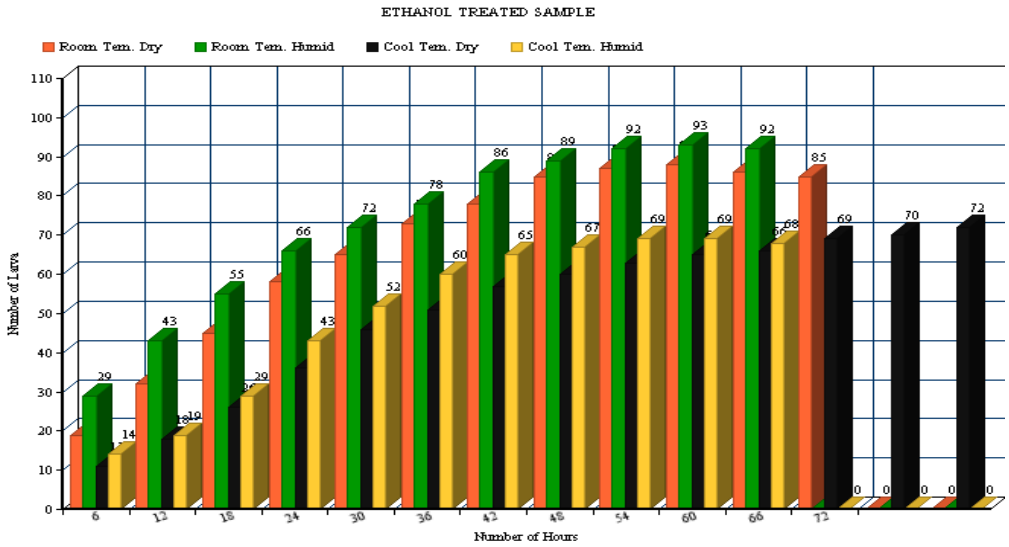

Fig 2: Sample Treated With Ethanol Shows Count of Larvae Taken Every 6 hrs After 1st Appearance Larva

3.9 Cannabis Treated Sample

3.9.1 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 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 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 (Table 17 and Table 18).

Table 17: Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
26th March	4 flies placed in jars, 1 fly dead
27th	No activity,1 fly dead
28th	Eggs laid,2 flies dead,
29th	1st instar,(2mm)
30th	2nd instar,(9mm)
31st	3rd instar,(16mm)
1st April	Pupae

Table 18: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 29th march

Hours	No. of larvae/pupae
6	25
12	39
18	49
24	59
30	70
36	78
42	82
48	85
54	89

60	85
66	89
72	94(*larvae/ pupae)*
78	90 *pupae*

3.10 Cannabis Treated Sample

3.10.1 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 (Table 19 and Table 20).

Table 19: Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
26th March	4 adult flies placed in jars, 1adult fly dead
27th	No activity,1 adult fly dead
28th	No activity , Eggs laid
29th	2 flies dead, 1st instar,(2mm)
30th	2nd instar,(9mm)
31st	3rd instar,(16mm)
1st April	Pupae

Table 20: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 29th march

Hours	**No. of larvae/pupae**
6	29
12	35
18	49
24	58
30	81
36	87
42	94
48	99
54	104
60	102 (*larvae/ pupae)*
66	101 (*larvae/ pupae)*
72	100 pupae

3.11 Cannabis Treated Sample

3.11.1 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 (Table 21 and Table 22).

Table 21: Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
26th March	**4** flies placed in jars,
27th	**N**o activity
28th	No activity ,2 fly dead
29th	Eggs laid, 2 fly dead,
30th	1st instar,(2mm)
31st	2nd instar,(9mm)
1st April	3rd instar,(16mm)
2nd	Pupae

Table 22: Shows Count of Larvae Taken Every 6 hours after First Appearance Larvae 30th march

Hours	No. of larvae/pupae
6	9
12	22
18	29
24	40
30	49
36	55
42	65
48	69
54	77
60	79
66	*79*
72	81(*larvae/ pupae)*
78	80 (*larvae/ pupae)*
84	79 *pupae*

3.12 Cannabis Treated Sample

3.12.1 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 (Table 23 and Table 24).

Table 23 Shows Observation Day Wise of the Jar Containing the Flies

Date of observation	Observation
2nd April	**4** flies placed in jars,
3rd	**N**o activity,2 adult fly dead
4th	No activity ,
5th	Eggs laid, 2 flies dead,
6th	1st instar,(2mm)
7th	2nd instar,(8mm)
8th	3rd instar,(15mm)
9th	Pupae

Table 24: Shows Count of Larvae Taken Every 6 hours After First Appearance Larvae 6th April

Hours	No. of larvae/pupae
6	12
12	25
18	39
24	52
30	58
36	71
42	74
48	80
54	81
60	85
66	86 (larvae/ pupae)
72	85 *pupae*

Sample treated with Cannabis shows count of larvae taken every 6 hrs after 1st appearance larva below in Figure 3.

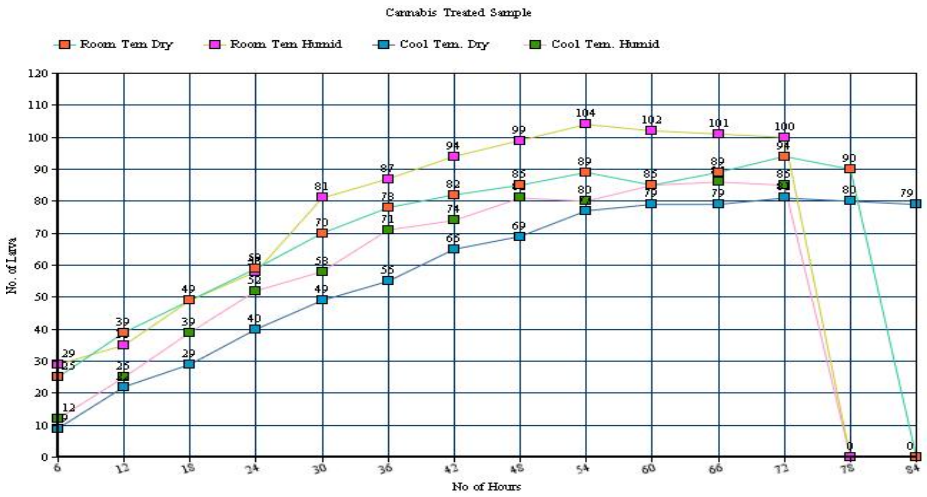

Fig 3: Sample Treated With Cannabis Shows Count of Larvae Taken Every 6 hrs After 1st Appearance Larva

4. Discussion:

Ethanol may be detected in the bodies of persons who have died regardless of the cause, but has its highest incidences in deaths from violent circumstances [16-17]. Ethanol is also one of the leading causes of death by poisoning [18-20]. Detecting alcohol in the tissues can provide important information involving the circumstances of an individual's death.

If antemortem ingestion of alcohol has occurred it could affect the development of insects that colonize and feed on the body. Ethanol caused significant differences in maggot length for third instars feeding on treated meat, compared to an untreated control in field conditions [21]. The presence of ethanol in tissue remains can affect the development of maggots and therefore estimates of PMI. There is also very little information concerning in larvae that feed on ethanol treated meat.

A large number of studies have been done on effects of temperatures on insect's life cycle. A few of them is [22-24]. They were reported that, *P. Sericata*, *P. Regina*, *Chrysomya rufifacies* (*Macquart*), and *Cochliomyia macellaria* (*F.*) development was slightly longer at fluctuating temperatures than at a mean, constant temperature. Mearns [25], Singh et. al.[26], Anderson[27] and Kamal[28] also investigated the effect of controlled temperature and humidity on

the life history, rate of development of thirteen species of flies, representing nine genera within the families *Calliphoridae*.

From India Singh et. al. [29] have emphasized the need to generate the much needed basic data so that this science can be put to proper use in India as well. This whole scenario prompted the author to undertake the present proposal. Singh et al have studied the forensic entomology in the Indian perspective, finding insects to be important forensic indicators. They studied the relationship between insects and corpse decomposition. Singh and Bharti enlisted the species of blowflies Order Diptera, Family *Calliphoridae*, Species *Chrysomya megacephala*, *Chrysomya rufifacies*, *Calliphora vicina, Lucilia Sericata, Lucilia illustris* available in the state of Punjab, which can be important from the forensic point of view.

54 cases of human dead bodies were studied by Dr. Akash Deep Aggarwal [30] for entomological evidence covering all the 5 seasons of the year recognized in the state of Punjab i.e. summer, rainy, autumn, winter and spring. The presence of *Calliphora vicina* was unique to the winter and spring seasons. *Chrysomya megacephala* and *Chrysomya rufifacies* were the two *Calliphorids*, which were found in all the seasons of the year. The duration of the decay process depended on climatic conditions and reflected yearly temperature changes. Corpses in summer and rainy season decayed at much faster rate than those in winter and spring. Warmer temperature in summer speeded up succession while low temperatures in winter retarded succession by slowing down the development of dipterous larvae.

Singh and Bharti also studied the nocturnal oviposition behavior of blowflies and confirmed the findings of Greenberg [31] that blowflies do lay eggs at night also and this factor should be taken into consideration while drawing conclusions on the basis of entomological evidence. Bharti and Singh [32] reported the occurrence of larval stages of blowflies during different stages of decay in rabbit carcasses. Bharti and Singh [33] studied the succession of insect communities in rabbit carrion in the state of Punjab. They reported 38 species of insects belonging to 13 families from different stages of carrion decay during various seasons of the year. Finding of feral derived from of *Chrysomya megacephala* (*Fabricius*) from India with an evolutionary novelty (Diptera, *Calliphoridae*) 2009 to determine the usefulness and applicability of forensic entomology in the Indian perspective.

The effects of temperature are a vital consideration in the calculation of the post mortem interval. Insect development is dependent on environmental temperature, where the higher the temperature, the faster the rate of development [34-35]. In many species, developmental rates were shown to be the same at natural, fluctuating temperatures, as at constant temperatures, when the temperature range is suitable and the constant temperatures represent the mean of the fluctuating temperatures [36-37]. Kulshreshtha and Chandra [38] observed that hatching will not take place or will be delayed by one or two days if weather is cold; and the warm weather, on the other hand, may advance the process.

5. Conclusion:

This study aims to develop an understanding of insect lifecycle, awareness of beneficial insect, understanding of forensic entomology and increase critical thinking application for student to further their interest in future career goals in entomology. Different temperatures have a variety of effects on the rate of insect development. As observed by Bayer [39] and later Levine [40] it was found that when the temperature was raised the larvae of blow flies developed at a higher rate with a rise in the number of off spring. The difference on temperature under which the study was conducted was 25^{o}C.

Also noted by Nabity *et al*. [41] it was clearly found that the cycle of colonization was affected by humidity and that blow flies thrive best in conditions were both a higher temperature and higher level of humidity. The density study of Slone Gruner [42] showed that some elevation of temperature occurs even at low temperature densities. An increased rate of development may

relate to temperature increases associated with the maggot feeding masses at higher densities. This study was confirmed with a similar trend being observed with the flies studied in this study.

This work is the one of the few attempts of this kind in India and the results will have direct application while using insects as evidence in forensic investigations. It will also form a model to undertake similar studies in different parts of the country besides increasing general awareness about forensic entomology. These studies shows that forensic investigators will have to take each of these variables into consideration apart from the normal time taken for the development of insect in its life cycle in order to give a more accurate estimate of PMI. Environmental influences, such as geographical location, climate, and weather conditions must be taken into consideration to the database of forensic literature. In conclusion, this research provides Entomological information and to determine the usefulness and applicability of forensic entomology in the Indian perspective. This type of information must be made available for the Indian species as well. In forensic investigations the scene of death is, of course, very commonly a house or some other indoor situation. Such locations would, therefore, be of particular interest as a subject for study

Insect development is dependent on environmental temperature, where the higher the temperature, the faster the rate of development [43-44]. To determine the post-mortem interval, where it cannot be determined by other methods, thereby helping in crime investigations Forensic entomology is a little used tool and a largely ignored field in India. Forensic Entomologist uses insects to help determine the cause, location and time of death of decomposed bodies [45]. They can be used to closely determine the time of death when other methods are useless. They can also show if a body has been moved after death [46]. In addition to estimating the post-mortem interval (PMI) insects that feed on carcasses may also represent a reliable specimen for toxicological analyses (Entomotoxicology).

In the above study the life cycle of *Chrysomya rufifacies* were studied under different temperatures and the effects of drugs on growth or colonization of *chrysomya rufifacies* at Noida, Uttar Pradesh, India. The use of growth development will depend on factors such as season, climate, and location of the corpse and treatment of the corpse. Furthermore, their rate of progress can be affected by a number of factors, including humidity, temperature, the presence or absence of clothing, burial and depth of burial[47]. Similar results were obtained in this study. 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 [48-51].

This study also 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, the samples grown in the presence of ethanol and cannabis showed a much faster growth rates. When the effects of the toxins on the growth rates were observed, a clearly distinct change was seen in the growth pattern. 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. Therefore all the parameter of this study has been proven that the differences in environmental conditions and presence of drugs affect the growth and colonization of *Chrysomya rufifacies*.

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7. References

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