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Effect of constant temperature (20 ^{0}C, 25 ^{0}C, 30 ^{0}C, 35 ^{0}C, 40 ^{0}C) on the development of the Calliphorid fly of forensic importance, *Chrysomya megacephala* (Fabricus, 1794)

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Abstract
The duration of the developmental stages of the insects depends on the temperature. The carrion flies are the best indicators to determine the post mortem interval in the criminal cases.
Chrysomya megacephala (Fabricus, 1794) is one of the Calliphorid flies having forensic and medical importance. In the present studies *C. megacephala* larvae were reared in incubator at 20, 25, 30, 35, and 40^{0}C separately in the incubator. The duration and the morphometric measurements of the developmental stages of *C. megacephala* for different temperatures were recorded.
Results obtained indicates that the developmental stages of *C. megacephala* grew normally up to 35 ^{o}C but at higher temperatures *Chrysomya megacephala* shows mortality, however the rate of the development of the developing stages increased with the increase in temperature. The life cycle was completed in about 14 days at 20 ^{0}C, 12 days at 25 ^{0}C, 10 days at 30 ^{0}C, 8 days at 35 ^{0}C and 7 days at 40 ^{0}C.

Keywords: Forensic, Calliphoridae, *Chrysomya megacephala*, temperature, humidity, indicators

1. Introduction
Medico legal forensic entomology includes arthropod involvement in events such as murder, suicide and rape, but also includes physical abuse and contraband trafficking. (Staerkeby 1997) Forensic entomology or medico-legal entomology can be defined as the study of insects associated with a human corpse in an effort to determine elapsed time since death (Catts and Goff 1992; Hall 2001; Zehner *et al*. 2004). When an unexpected death occurs without any witness or superficial evidence, estimation time of the death becomes a major concern.
The major contribution normally made by forensic entomologists in death investigations is the estimation of time between death and corpse discovery. This is referred as post-mortem interval (PMI) (Amendt *et al*. 2006).
Insects recovered from human cadavers, predominantly blowfly larvae, can provide information on the conditions experienced by a body following death (Donovan *et al.* 2006). Forensic entomology is the name given to any aspect of the study of insects and their arthropod counterparts that interacts with legal matters (Hall and Doisy 1993).
Larvae of carrion flies, especially blowflies are by far the most common type of insect evidence collected during a death investigation (Cattsand Goff 199; Zehner *et al*. 2004). These blowflies are the first to arrive at the dead body due to the odor of fresh blood, especially blood from open wounds and body fluid (Anderson *et al*. 2000) [5].
Blow flies are distributed worldwide and cause medical problems and losses to animal industry (Zumpt. 1965; Greenberg, 1971, 1973; Kuhihorn, 1983; Ghandour, 1988). To determine time since death, considerations of the critical factors affecting the rate of decomposition are important. These factors include location of the body, temperature, general climate of year, insect activity, animal activity in the area and the amount of rainfall (Nafte, 2000). Temperature is the most important factor affecting developmental rate (Myskowiak and Doums, 2002).
The succession of arthropods development is mostly affected and influenced by temperature and humidity (Grassberger and Reiter 2001; Ames and Turner 2003) [20, 3]. In warmer temperature and high moisture condition, insects have been known to grow faster. The opposite conditions have also been noted to retard insect growth significantly (Anderson *et al*. 2000) [5].

In this study the effect of five different temperatures on the duration of developmental stages of the life cycle on *Chrysomya Megacephala* has been studied to prepare the data for the PMI determination.

2. Materials and Methods

Adult flies of *Chrysomya megacephala* were collected from different regions of Osmanabad district (MS) India, and reared in the laboratory in the rearing box feeding daily on fresh beef liver and honey water. *C. megacephala* eggs from the room temperature were collected and transferred into rearing containers. All of the larvae were incubated inside the incubator set at different constant temperatures (20, 25, 30, 35, and 40 ^{0}C). At each temperature, eggs, larvae, pupae and adult flies grown on fresh liver were observed daily. The length and weight of the larvae and pupae were also measured. The duration of different life cycle stages at various temperature conditions were determined. The temperature and humidity of incubator were recorded by Hygro-thermometer.

3. Observation and Results

3.1 Results

The lowest temperature used for this study was 20 °C. The life cycle of the fly completed in 15 days. Table No.1 shows record of different parameters of the *Ch. megacephala* observed during the study. Fly laid eggs when maximum recorded temperature was 29 °C and minimum was 23.4 °C. Humidity inside the incubator was 60.05 which was low as compared to normal surrounding temperature. On fifteenth day adult emerged from the puparium and the recorded temperature was 38.8 °C maximum and minimum 23 °C. Humidity inside the incubator was 70.When temperature raised to 25 °C, life span of the fly completed in 12 days only. Due to increased temperature life span of the fly completed three days before. Table no.2 shows recorded parameters during the study. Table 3 shows a further increase in the temperature by 30 °C. At this stage larva reaches adult in 10 days. Due to increased temperature life cycle of the fly reduced by two days. Table no.4 shows the effect of 35 °C temperatures on the development of *Ch. megacephala*. At this temperature *Ch. Megacephala* has completed its life cycle in seven days. As compared to table no.1, 2, 3 table no.4 shows record of rapid development of the *Ch. megacephala*. There is slight increase in the length of the maggots. At 40 °C *Ch. megacephala* shows slight mortality. Flies cannot tolerate high temperature. Somehow *Ch. megacephala* has completed its life cycle in 7 days. From above observations it is clear that 35 °C is the suitable temperature for the development of *Ch. megacephala*.

3.2 Observation Tables

Table 1: Effect of temperature on life cycle of *Chrysomya Megacephala* at 20±1^{0}C

Sr. No	Date	Stage	Time	Weight in mg.	Length in mm	Humidity in incubator	Normal Temp. ^{0}C		Normal Humidity	
							Max	Min.	8.30am	5.30pm
1	27.07.15	Eggs	12.30pm	-	1.2	60.05	29	23.4	73	68
2	28.07.15	I Instar	10am	01	3.1	69	29.4	22.6	77	62
3	29.07.15	IIInstar	10am	04	4	68.02	28.5	22.2	77	90
4	30.07.15	IIIInstar	10am	12	8	69.03	30.2	22.7	85	65
5	31.07.15	Prepupa	10am	24	10	60.08	31.4	22.5	84	62
6	01.08.15	Prepupa	10am	32	11	68.02	33	22	84	57
7	02.08.15	Prepupa	10am	34	11	71	31.7	23	84	57
8	03.08.15	Pupa	10am	32	1	69.03	32	22.6	79	53
9	04.08.15	Pupa	10am	--	--	69.07	24	23.5	88	97
10	05.08.15	Pupa	10am	--	--	72	23.6	21	97	98
11	06.08.15	Pupa	10am	--	--	69	23.6	21.8	100	98
12	07.08.15	Pupa	10am	--	--	69.05	29	21.7	92	71
13	08.08.15	Pupa	10am	--	--	69.04	30.4	23	85	71
14	09.08.15	Pupa	10am	--	--	69	31.4	22.4	87	51
15	10.08.15	Adult	9am	--	--	70	30.8	23	85	72

Table 2: Effect of temperature on life cycle of *Chrysomya Megacephala* at 25±1 °C

Sr.no	Date	Stage	Time	Weight in mg.	Length in mm	Humidity in incubator	Normal Temp. ^{0}C		Normal Humidity	
							Max	Min	5.30pm	8.30am
1	27.07.15	Eggs	4pm	0.000	0.12	65	29	23.4	73	68
2	28.07.15	I Instar	12.30pm	0.001	0.31	68	29.4	22.6	77	62
3	29.07.15	II Instar	10am	0.004	0.5	70	28.5	22.2	77	90
4	30.07.15	III Instar	10am	0.012	0.8	64	30.2	22.7	85	65
5	31.07.15	Prepupa	10am	0.024	1.1	64	31.4	22.5	84	62
6	01.08.15	Prepupa	10am	0.032	1.2	66	33	22	84	57
7	02.08.15	Prepupa	10am	0.034	1.2	68	31.7	23	84	57
8	03.08.15	Pupa	10am	0.032	1	70	32	22.6	79	53
9	04.08.15	Pupa	10am	--	--	69	24	23.5	88	97
10	05.08.15	Pupa	10am	--	--	66	23.6	21	97	98
11	06.08.15	Pupa	10am	--	--	75	23.6	21.8	100	98
12	07.08.05	Adult	11am	--	--	70	29	21.7	92	71

Table 3: Effect of temperature on life cycle of *Chrysomya Megacephala* at 30±1 °C

Sr.no.	Date	Stage	Time	Weight in mg.	Length in mm	Humidity in incubator	Temp Normal^{0}C		Humidity Normal	
							Max	Min	8.30am	5.30pm
1	14.08.15	Eggs	1.30pm	0.000	0.12	58	29.8	22.8	92	73
2	15.08.15	I Instar	10am	0.001	0.31	59	30.4	22.8	86	74
3	16.08.15	II Instar	10am	0.005	0.5	62	31.6	22.5	84	66
4	17.08.15	III Instar	10am	0.014	0.9	64	31.2	22.9	89	70
5	18.08.15	Prepupa	10am	0.025	1.2	60	32	23.2	84	60
6	19.08.15	Prepupa	10am	0.033	1.4	71	33	24.6	78	63
7	20.08.15	Pupa	10am	0.034	1.3	68	32.4	23.4	75	48
8	21.08.15	Pupa	10am	0.035	1.2	69	32	22.4	72	56
9	22.08.15	Pupa	10am	0.035	1.2	70	31.4	22.4	84	53
10	23.08.15	Adult	10am	--	--	72	32.4	21.8	77	49

Table 4: Effect of temperature on life cycle of Chrysomya Megacephala at 35±1 °C

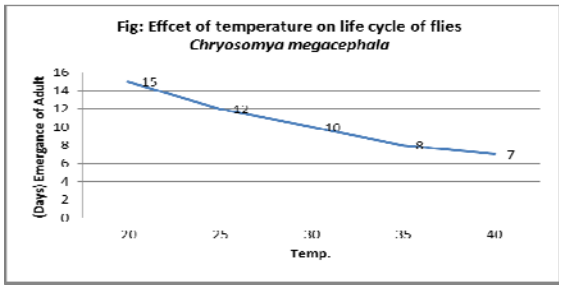
Sr. No	Date	Stage	Time	Weight in mg.	Length in mm	Humidity in incubator	Normal Temp. ^{0}C		Normal Humidity	
							Max.	Min	8.30am	5.30pm
1	14.08.15	Eggs	11am	0.000	0.12	78	29.8	22.8	92	73
2	15.08.15	I Instar	10am	0.001	0.35	75	30.4	22.8	86	74
3	16.08.15	II Instar	10am	0.005	0.62	70	31.6	22.5	84	66
4	17.08.15	III Instar	10am	0.014	0.9	78	31.2	22.9	89	70
5	18.08.15	Prepupa	10am	0.018	1.2	78	32	23.2	84	60
6	19.08.15	pupa	10m	0.019	1.2	80	33	24.6	78	63
7	20.08.15	pupa	2pm	--	--	79	32.4	23.4	75	48
8	21.08.15	Adult	9am	--	--	78	32	22.4	72	56

Table 5: Effect of temperature on life cycle of *Chrysomya Megacephala* at 40±1 °C

Sr. No	Date	Stage	Time	Weight in mg.	Length in mm	Humidity in incubator	Normal Temp.^{0}C		Normal Humidity	
							Max	Min	8.30am	5.30pm
1	29.08.15	Eggs	1pm	0.000	0.12	84	31	23.1	87	66
2	30.08.1	I Instar	10am	0.001	0.35	86	31.4	22.8	84	66
3	31.08.15	II Instar	10am	0.005	0.6	79	31.4	23	84	61
4	01.09.15	III Instar	10am	0.014	0.8	80	32.2	23.6	84	58
5	02.09.15	Prepupa	10am	0.018	1.1	77	31.4	22.5	89	50
6	03.09.15	Prepupa	11am	0.020	1.1	88	33.2	21.8	66	50
7	04.09.15	Adult	10am	--	--	84	32.8	22.2	76	95

Table 6: Time duration required to complete the life cycle stages of *Chrysomya megacephala* at different temperatures

Days	20 °C	25 °C	30 °C	35 °C	40 °C
01	Eggs	Eggs	Eggs	Eggs	Eggs
02	IInstar	I Instar	I Instar	I Instar	I Instar
03	IIInstar	II Instar	II Instar	II Instar	II Instar
04	IIIInstar	III Instar	III Instar	III Instar	III Instar
05	Prepupa	Prepupa	Prepupa	Prepupa	Prepupa
06	Prepupa	Prepupa	Prepupa	pupa	Pupa
07	Prepupa	Prepupa	Pupa	pupa	Adult
08	Pupa	Pupa	Pupa	Adult	
09	Pupa	Pupa	Pupa		
10	Pupa	Pupa	Adult		
11	Pupa	Pupa			
12	Pupa	Adult			
13	Pupa				
14	Adult				



Graphical representation of the effect of constant temperature on *Chrysomya megacephala*

4. Discussion

The results demonstrated that temperature plays a major role in influencing and controlling the insects' activity. Entomological evidences found in criminal scene around the corpse should be collected and preserved according to medico-legal standard procedures (Haskell, *et al*. 1997) which can be compared with the known data for the correct PMI determination.

The results demonstrated that temperature plays a major factor in influencing and controlling the insects' activity. Davidson (1944) [13] has studied effects of temperature on developmental time of blow fly life cycles and defined relationship between temperature and rate of development of insects at constant temperatures.

In this study the lowest temperature studied was 20ºC. At this temperature, development of the insect was the slowest compared to other temperature as noted by Payne *et al*. (1965) [23] and Smith (1986) [24]. *Chrysomya Megacephala* also known as the oriental latrine fly is a species of medical importance (Sukontason, *et al.* 2003) and playing an important role in forensic cases (Smith, 1986) [24]. Wells and Kurahashi (1994) have studied the development of *C. megacephala* at 27 °C and observed that there were differences in the length of post feeding larvae and attributed these differences to their sensitiveness to environmental conditions. Milward-de-Azevedo *et al*. (1996) [22] have analyzed the influence of temperature onthe postembryonic development of *Chrysomya megacephala* in an incubators regulated at 18 °C, 24 °C, 30 °C and 35 °C.

In warmer temperature and high moisture condition, insects have been known to grow faster while opposite conditions have also been noted to retard insect growth significantly (Anderson *et al*. 2000) [5]. Insect development is dependent on environmental temperature, higher the temperature, faster the rate of development. (Anderson 2000, Brewer 2001) [5, 6]. Wang *et al*. (2001) [29] had studied chronology of development within puparium of *C. megacephala* at different constant temperatures which could help in estimation of postmortem interval.

Wang *et al*. (2002) [30] had further observed the effect of temperature on the body length change of C. megacephala that has implications to forensic entomology.

Silva *et al*. (2004) have analyzed the theoretical population dynamics of C. megacephala kept at constant temperatures using density-dependent mathematical model, with parametric estimates of survival and fecundity in the laboratory.

Gabre *et al*. (2005) have also provided a life table of *C. megacephala* reared at 26 °C in the laboratory.

The results demonstrated that temperature plays a major factor in influencing and controlling the insects' activity, oviposition rate and as well as their overall development as was reported previously (Marinho *et al*. 2006).

Bharti *et al*. (2007) [8] had observed the effect of temperature on the development of *C. megacephala* reared in the laboratory at four constant temperatures (22 °C, 25 °C, 28 °C, 30 °C).

Mohd. *et al.* (2007) found same results in his study. He C. megacephala larvae inside a special incubator with temperature adjusted to 27, 30, 33, 36 and 39 °C separately. He found that the larvae reached adulthood at 27 °C. At 39 °C larvae grew much rapidly, although some pupae developed into adults as early as day 2 at 39 °C, they were not healthy and died.

Niederegger *et al*. (2010) have compared the development of larvae of different forensically important flies under constant low, medium and high temperatures as well as under daily fluctuating temperatures in climatic chambers.

In Riyadh, Saudi Arabia, Amoudi *et al.* (1994) [4] studied the developmental rate of Parasarcophaga (Liopygia) ruficornis (Diptera: Sarcophagidae), at constant laboratory temperatures. Effect of fluctuation of temperature on development of Calliphoridae, flies Protophormia terraenovae was reported at 4-28 and 9-23 °C to their mean constant temperature,16 °C and found that generally development at the greater fluctuation was fast and at the constant temperature was slow and found similar percentages of development time in each stage. The effect of summation rate is suspected to have caused this difference in development rate because fluctuations above the mean enhance the rate comparatively more than temperatures below the mean can lower the rate (Warren and Anderson, 2013) [31].

Fahad and Zambre (2015) have studied the effects of temperature on the development of Calliphorid fly of forensic importance *Chrysomya* at Constant temperature. He observed that low temperature not only delays the duration of life cycle but also have impact on the morphological parameters like length, width and weight. At normal room temperature in rainy season the length, width and weight of second instar were 8.4 ± 0.16 mm 1.8 ± 0.66 mm and 23.2 ± 0.37 mg respectively. While at low temperature, 10 ± 0.05 °C the length, width and weight of second instar larvae were 6.8 ± 0.16 mm 1.4 ± 0.08 mm and 18.5 ± 0.67 mg. Thus in rainy season the duration required from laying of eggs to reaching the second instar was 77 hrs. (3.21 days), but at constant low temperature same period was 153 hrs. (6.38 days). In rainy season the total larval duration was 143 hrs. (5.96 days) at room temperature, while at low temperature it was 343 hrs. (14.29 days). The pupal stage remained for 122 hrs. (5.08 days) at room temperature in rainy season while at low temperature (10 °C) it was 266 hrs. (11.08 days).

5. Conclusion

It is observed that temperature affects the life cycle of the flies. Low temperature increases the duration to complete the life cycle whereas high temperature decreases the duration to complete the life cycle.

Variation in temperature and humidity influence growth and indirectly influence the estimation of time since death. Thus to ensure a more accurate estimation, history of surrounding temperature and humidity in the location where body was found must be taken into consideration.

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