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# EFFECT OF SEASONAL TEMPERATURE VARIATIONS ON THE LIFE CYCLE DURATION OF FORENSICALLY IMPORTANT CALLIPHORIDAE FLY *CHRYSOMYA INDIANA* (ABD ALGALIL & ZAMBARE 2016)

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## ABSTRACT

*Chrysomya indiana* (Abd Algalil & Zambare, 2016) is one of the forensically important Calliphoridae flies discovered in India. Larvae were recovered from dead body of a street dog in the campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

This study was conducted to determine the effect of temperature variations and relative humidity on the life cycle and the morphological parameters of *C. indiana* in different seasons. The species was fed with fresh liver of buffalo and reared in the different seasons at normal condition in the laboratory.

The obtained results showed that in rainy season life cycle, it was completed in 275 hrs (11.46 days) when and in winter season, it was completed in 330 hrs (13.75 days). While in summer season, it was completed in 218 hrs (9.08 days). Morphological parameters of different stages also varied from season to another.

Statistical analysis at level of $P \leq 0.05$ has shown significant variation in the PMI and the morphological parameters.

Also the obtained results have shown significant variation in the humidity among the stages and among the seasons. While the temperature shown significant variation among the seasons only; but there was no significance variation was observed among the stages. So, this can prove that the humidity have a great effect on the life cycle duration of the *C. indiana*.

**Keywords: Calliphoridae, *Chrysomya indiana*, Life Cycle, Temperature and Humidity**

## INTRODUCTION

Forensic entomology or medico-criminal entomology mainly deals with the carrion insects and other arthropods as a tools to aid a legal investigations [1-2]. The close relation between insects and remains (animal/ human) and the use of insects in medico-criminal investigations help the forensic investigator to solve the crime puzzles and estimation of postmortem interval [3]. The most common carrion larvae recovered from the different crimes and human cadavers are blowflies (Calliphoridae family) [4-5].

A new blowfly species *Chrysomya indiana* (Abd Algalil & Zambare 2016) first discovered and described in Aurangabad City, Maharashtra State, India [6-7] and reported with Animal Discoveries 2016 published Zoological Survey of India [8]. This species recovered from animal cadaver of street dog.

Blowflies of the genera *Chrysomya* (Diptera: Calliphoridae) are considered as a medical and economic importance since it is a myiasis producing agent in humans as well as animals. Calliphoridae flies are also important in forensic entomology since they can aid a criminal investigation [9-11]. *Chrysomya indiana* is like other Calliphoridae species of forensically importance and it could be use for Postmortem Interval (PMI) determination if it is recovered from the crime scene.

There are many factors that can affect the activity of carrion flies and their life cycle duration. These factors are temperature, humidity, geographical location, seasons and habitat [12-14]. Therefore, determination of these factors and their interaction with the insect activity and life cycle are the most active arena of research in forensic entomology [6].

In this study, the effect of normal seasonal variation of temperature and humidity (at laboratory) on the life cycle of *Chrysomya indiana* was researched so as to provide database for the application of this species in forensic practice.

## MATERIAL AND METHODS

*Chrysomya indiana* larvae were collected from the dead body of street dog

*Canis lupus familiaris* at the Botanical Garden of the campus of Dr Babasaheb Ambedkar Marathwada University Reserve (19.9047N, 75.3102E), Aurangabad City, Maharashtra State, India. The collected larvae were reared in the laboratory under normal laboratory condition. Maggot culture was provided with fresh liver of buffalo as a food till the 3rd instar started the post feeding stages (prepupae stage), at this stage maggots used to leave the food and start the pupariation stage. Prepupae were kept in 500 ml beakers containing dry soil which is required for the pupation. Adult flies which emerged out from pupae were reared in rearing boxes of size 22× 12 × 10 inches in dimensions (length × width × depth respectively) [5]. The eggs separated immediately after depositing from one female to maintain a pure culture of one species. From the pure culture, larvae and adult were dissected for identification with the help of stereo-zoom microscope (ERMA Optical works, Tokyo, No. 44883) and light microscope (Magnus Trinocular Microscope MLX-DX, Olympus -India PVT. LTD. No. 4B525145). *C. indiana* was identified genetically and morphologically according to the previous studies [6, 7].

**Experimental Design**

In these experiments, after maintaining the pure culture of *Chrysomya indiana*, eggs were directly collected after laying with the help of fine brush and reared in summer, rainy and winter seasons at laboratory condition, to insure the same environmental condition at laboratory with12hrs Dark and 12hrs light; three experiments were conducted at the same time. Three groups of 80 larvae separately transferred into three glass beakers 500ml capacity; each group of larvae were daily fed on 50gm of buffalo`s fresh liver till pupation. At the pupation stage, larvae were placed in small plastic beakers 1L capacity with 200 gm of dried soil which is necessary for the pupation. Life cycle duration of different stages and the morphological parameters were daily recorded. The temperature and relative humidity were also recorded by using Hygrothermometer clock. Seasonally, this experiment was repeated three times.

**Statistical Analysis**

Using the excel sheet statistical analyses were performed, data were analyzed statistically by using Two-ways analysis of variance (ANOVA) test and significance level at $P \leq 0.05$ was used [5].

**RESULT**

This species is holometabolous like other species of same genera and the stages known as eggs (pre-feeding stage), maggots

as 1st, 2nd and 3rd instar larvae (feeding stages), prepupae and pupae (post feeding stages). Life cycle duration of *Chrysomya idiana* has been studied at laboratory condition at different season.

The results have shown significant variations in total PMI duration at different seasons in the level of P ≤ 0.05. The long duration observed in winter season 330hrs, followed by rainy season 275hrs and the short duration was in summer season 218hrs. Similarly, significant variation also observed in the developmental duration of different sages at level of P ≤ 0.05 (Table: 1). Similarly, the data obtained shown significant fluctuation in temperature among seasons, and there was no significant fluctuation at level of P ≤ 0.05 among the stages was shown (Table: 2). While the relative humidity shown significant among the seasons and the stages (Table: 3). The morphological parameters (Length, width and weight) of each stages shown significant variation in different seasons at level of P ≤ 0.05, the big sizes of different stages were in rainy season followed by summer season and the smallest sizes of different stages were observed in winter (Table: 4-6).

**Table 1: PMI in hours of different developmental stages in different Seasons**

| Developmental Stages | PMI in Hours | | | |
|---|---|---|---|---|
| | Summer | Rainy | Winter | |
| Eggs | 17± 0.50 | 24±0.25 | 29±1.10 | P-value= 1.47E-07 |
| 1st instar | 37±1.30 | 48±1.30 | 61±1.15 | |
| 2nd instar | 61± 1.15 | 76±1.10 | 99±1.50 | |
| 3rd instar | 88±1.10 | 110±2.40 | 141±2.01 | |
| Prepupae | 116±2.20 | 146±1.15 | 188±1.73 | |
| pupae | 218±1.5 | 275±2.5 | 330±2.25 | |
| Adult | P-value= 0.0021 | | | |

*** Means no significant variation at level P ≤ 0.05.**

**Table 2: Temperature fluctuations in different seasons**

| Developmental Stages | Average temp. (°C) | | | |
|---|---|---|---|---|
| | Summer | Rainy | Winter | |
| Eggs | 30.6 | 28.5 | 22.5 | P-value= 0.74 * |
| 1st instar | 31.4 | 28.5 | 22.5 | |
| 2nd instar | 30.6 | 27.6 | 20.6 | |
| 3rd instar | 32.3 | 27.2 | 20.6 | |
| Prepupae | 32.7 | 27.3 | 19.6 | |
| pupae | 31.1 | 26.5 | 20.6 | |
| Adult | 32.7 | 25.6 | 22.3 | |
| P-value= 3.55E-09 | | | | |

**Table 3: Humidity variations in different seasons**

| Average Humidity (%) | | | | |
|---|---|---|---|---|
| **Developmental Stages** | **Summer** | **Rainy** | **Winter** | |
| **Eggs** | **22** | **65** | **30** | **P-value= 0.0279** |
| **1st instar** | **22** | **65** | **29** | |
| **2nd instar** | **21** | **59** | **30** | |
| **3rd instar** | **20** | **59** | **31** | |
| **Prepupae** | **21** | **54** | **28** | |
| **pupae** | **20** | **54** | **26** | |
| **Adult** | **19** | **54** | **22** | |
| **P-value= 5.2E-12** | | | | |

**Table 4: Length of different developmental stages in different seasons**

| Developmental Stages | Length (mm) | | | |
|---|---|---|---|---|
| | **Summer** | **Rainy** | **Winter** | |
| **Eggs** | **1.5 ± 0.06** | **1.5 ± 0.06** | **1.4 ± 0.04** | **P-value= 9.87E-9** |
| **1st instar** | **5.2 ± 0.23** | **6.2 ± 0.64** | **4.3 ± 0.08** | |
| **2nd instar** | **8.6 ± 0.23** | **9.5 ± 0.23** | **7.5 ± 0.23** | |
| **3rd instar** | **13.5 ± 0.24** | **15.4 ± 0.20** | **10.5 ± 0.21** | |
| **Prepupae** | **11.4 ± 0.06** | **12.2 ± 0.18** | **9.2 ± 0.13** | |
| **pupae** | **8.5 ± 0.23** | **9.6 ± 0.20** | **8 ± 0.22** | |
| **Adult** | **8.2 ± 0.12** | **9.3 ± 0.16** | **7.6 ± 0.33** | |
| **P-value= 0.000737** | | | | |

**± Standard deviation of five values**

**Table 5: Width of different developmental stages in different seasons**

| Developmental Stages | Width (mm) | | | |
|---|---|---|---|---|
| | **Summer** | **Rainy** | **Winter** | |
| **Eggs** | **0.4 ± 0.06** | **0.4 ± 0.06** | **0.4 ± 0.02** | **P-value= 8.25E-12** |
| **1st instar** | **1.5 ± 0.08** | **1.8 ± 0.84** | **1.3 ± 0.06** | |
| **2nd instar** | **2.5 ± 0.26** | **3 ± 0.15** | **2 ± 0.10** | |
| **3rd instar** | **4 ± 0.11** | **4.4 ± 0.66** | **3.8 ± 0.01** | |
| **Prepupae** | **3.8 ± 0.33** | **4.2 ± 0.45** | **3.6 ± 0.08** | |
| **pupae** | **3.6 ± 0.07** | **4 ± 0.24** | **3.3 ± 0.19** | |
| **Adult** | **3.3 ± 0.17** | **3.6 ± 0.33** | **3 ± 0.23** | |
| **P-value= 5.16E-05** | | | | |

**± Standard deviation of five values**

**Table 6: Weight of different developmental stages in different seasons**

| Developmental Stages | Weight (mg) | | | |
|---|---|---|---|---|
| | **Summer** | **Rainy** | **Winter** | |
| **Eggs** | **0.30 ± 0.07** | **0.40 ± 0.09** | **0.27 ± 0.21** | **P-value= 1.81E-09** |
| **1st instar** | **12.5 ± 0.11** | **21.5 ± 0.24** | **8 ± 0.01** | |
| **2nd instar** | **30.2 ± 0.14** | **37.2 ± 0.38** | **25.5 ± 0.12** | |
| **3rd instar** | **60.2 ± 0.22** | **70.5 ± 0.08** | **50.4 ± 0.37** | |
| **Prepupae** | **54.5 ± 0.08** | **60.1 ± 0.12** | **40.3 ± 0.33** | |
| **pupae** | **42.6 ± 0.27** | **46.6 ± 0.45** | **33.5 ± 0.08** | |
| **Adult** | **30.5 ± 0.19** | **36.5 ± 0.25** | **27.2 ± 0.12** | |
| **P-value= 0.000132** | | | | |

**± Standard deviation of five values**

**DISCUSSION**

In rainy season, total time spent in feeding stages (1st, 2nd ad 3rdinstar) was 86 hrs (3.58 days) and in winter season feeding stages spent 112 hrs (4.67 days), while in summer season, was 71 hrs (2.96 days),. Similarly, the time spent in post feeding stages in rainy season was 165 hrs (6.88 days) and in winter season spent 189 hrs (7.88 days), while in summer season was 130 hrs (5.42 days). The time spent in feeding and post feeding stages varied in different seasons according to the species and the effect of temperature and humidity. These results prove that the high temperature accelerates the development of the larvae and the low temperature slows down the development.

Similar result reported on developmental time of forensically important blow fly species *Phormia regina* (Meigen, 1826) at different constant minimum and upper threshold temperatures (8-32 °C) the study has shown that no development occurred at 12 °C and below but in 14 °C total life cycle was of 45 days and on 26 °C total life duration was 13 days, while in 32 °C total life cycle was of 11.3 days [15]. Our obtained results also in agreement with another study on the developmental rate of forensically important species *Chrysomya albiceps* at different constant temperature 15, 20, 25, 30 and 35 °C and reported at 15 °C all pupae failed to develop to adult but the total life cycle at 20 °C was about 18.5 days, while at 25, 30 and 35 °C total life cycle were 14.1, 9.8 and 9.2 days respectively, these results support that shortest life cycle was at high temperature and long duration at low temperature [16].

Another study on the total life cycle duration of *C. saffranea* in different seasons strongly supported our obtained result, the study reported life cycle duration of *C. saffranea* in rainy season completed in 259 hrs (10.79 days) when the average temperature ranged from 25.6 - 28.9°C and average humidity ranged from 50 - 65% and in winter season was completed in 341 hrs (14.21 days) when the average temperature and humidity ranged from 17.8 – 24.4 °C and 17 - 28 % respectively, while in summer season was completed in 220 hrs (9.17 days) when the average temperature ranged from 30.5 - 33.2°C and average humidity ranged from 12 - 19 %, [15].

Likewise, our results in current study are supporting previous study on *L. sericata* when it was reared in different temperatures 15, 17, 19, 20, 21, 22, 25, 28, 30 and 34 °C, the shortest life cycle was 259 hrs in 34 °C while the longest duration was 842 hrs in 17

°C. But in 15 °C the period recorded up to pupation was 340 and there are no records of emergence of adult in this lowest temperature [17].

Similarly, Grassbereger and Reiter, [17] reported that the average minimum duration of different stages of *L. sericata* at 22 °C were 17 hrs as incubation period of eggs and 19, 26, 46 hrs for 1st , 2nd and 3rd instar larvae while prepupae and pupae were 94, and 137 hrs respectively and the total lie cycle was completed in 339 hrs while Greenberg [18] reported average minimum duration of different stages of *L. sericata* at 22 °C was 23 hrs as incubation period of eggs and 27, 22, 22 hrs for 1st , $2^{nd}$ and $3^{rd}$instar larvae while for prepupae and pupae were 106, and 143 hrs respectively and the total life cycle was 343 hrs with very small variation it may be due to the other factor like relative humidity.

Another supporting study reported that the total life cycle of *C. rufifacies* in summer season was completed in 241 hrs (10.04 days) when the average temperature ranged from 32 - 35.7 °C and average humidity ranged from 19 - 30 % and in rainy season completed in 275 hrs (11.46 days) when the average temperature ranged from 26.1- 29.2 °C and average humidity ranged from 51 - 56 %, while in winter season was completed in 318 hrs (13.25 days) when the average temperature and humidity ranged from 19.8 – 24.3 °C and 20 - 29 % respectively [19].

Sukontason *et al*. [12] studied the developmental time of *C. rufifacies* under various natural temperatures and found many variations, in lowest range of temperature 22.7 °C, the time spent from the newly deposited eggs up to pupariation was 186 hrs and in 23.8 °C spent 192 hrs, but in 25 °C range of temperature it took 132 hrs while in 27 °C found that the stages from egg laying to puparition took different readings 96, 132 and 180 hrs in the same temperature, again in the range of temperature 28 °C developmental time was 120 and 180 hrs, while in 31 °C range temperature time spent was 120 hrs, and conclude that the developmental rate at 25.2°C, it took 132 hrs, which was less than 170 hrs at 25.0°C. At the higher temperature of 31.3°C, which was the highest natural temperature, the growth until pupariation was ≈120 hrs. This time was slightly longer than 116 hrs at 32.2°C. In another study Byrd and Butler [20] reported with a mean temperature of 21.1°C, took ≈174 hrs, which is close to the previous result of [12]. The records in both of previous studies are slightly different from the results obtained in current study as it reported the

shortest of life cycle in summer season followed by rainy season and winter season which may be because of other natural factor like the climatic condition (humidity) or geographical region. Greenberg, [18] reported that the developmental times from the depositing of the eggs up to the eclosion might be differing in various regions of the world.

Another study on *C. megacephala* at a controlled temperature of 27°C and light conditions of 16 hrs Light: 8 hrs Dark reported that the development time from eggs laying to pupariation was 144 hrs [21], while Sukontason et al. [12] reported development time of *C. megacephala* in similar range of temperature at a mean of 27°C in June, July, and September in from newly hatched larvae until pupariation 96, 108, and 168 hrs, respectively. They also reported that the variables in the development time in April, May and January were 84, 108 and 144 when the temperatures were 31, 28 and 22.7°C respectively.

Joy *et al*., [22] reported that the carcass decomposition proceeded more rapidly in the presence of higher ambient temperatures and sunlit carcasses decomposed faster than shaded ones.

Our results in current study are in agreement with the previous study which was reported that the total life cycle of *C. megacephala* in summer season was completed in 232 hrs (9.67 days) when the average temperature ranged from 32.7 - 35.8 °C and average humidity ranged from 19 - 26 % , in rainy season completed in 265 hrs (11.04 days) when the average temperature ranged from 25.9 - 29.1 °C and average humidity ranged from 54 - 65 %, while in winter season was completed in 328 hrs (13.67 days) when the average temperature and humidity ranged from 13.4 - 18.1 °C and 20 - 30 % respectively[6].

Larval Development of carrion flies has been reported to be accelerated at higher temperatures in both laboratory [18, 23-24] and field experiments in shaded and sunlit [25-26]. Relative humidity also play an important role in the developmental time of insects, when the humidity very high the developmental time will be delay and the larvae will not pupate, even if larvae pupate in very high moisture condition, adult will fail to emerged from the pupae [21].

**CONCLUSION**

The current study assured that the favorable temperature for the development of *C. indiana* ranged between 28.5 and 25.6 °C and the favorable humidity ranged between 54 to 65 %. Higher temperature leads to water evaporation from the corpse and

caused dryness of the tissues and the time will delay; the size of the different stages will be small. The low temperature decelerates the developmental time and reduces the size of the different stages. High temperature can speed up the development and also reduce the size of the different stages.

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