

# Studies on the Statistical Analysis and Impact of Seasonal Temperature and Relative Humidity Variation on the Development of a Flesh Fly *Sarcophagadux* (Thomson 1869)(Diptera Sarcophagidae)

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**How to cite this article:** B.Suri Babu, Arvind Agarwal, Devinder kumar et. al. Studies on the Statistical Analysis and Impact of Seasonal Temperature and Relative Humidity Variation on the Development of a Flesh Fly *Sarcophagadux* (Thomson 1869)(Diptera Sarcophagidae). Medico Legal Update / Vol. 24 No. 4, October-December 2024.

## Abstract

**Background:** The fleshfly *Sarcophaga dux* (Diptera;Sarcophagidae) is found in Indian Sub continent is of limited forensic significance as its poorly known larval development relationship with temperature and relative humidity.

**Objective:** The objective of this study was to determine the effect of the temperature and relative humidity in the larval development of this fly in two seasons, Summer and Rain, so that the results of this study may enabling the forensic use of this fly in the estimation of Post Mortem Interval(PMI).

**Material and Method:** The present study was carried out in the outer premises of the Regional Forensic Science Laboratory Building, Jagdalpur, Bastardist,Chhattishgarh, India. The fresh meat of male goat was used for rearing of larvae. The effect of temperature and relative humidity on the larval developmental time of this fly in two seasons, Rainy and Summer were studied in normal condition. Statistical analysis of variance was performed and standard significance level of  $P < 0.05$  was taken as standard.

**Results:** The results of this study shown that in Rainy season it was completed in 264 h (11 days) when the average temperature ranged from 21.8°C to 24.5°C and the average humidity ranged from 79.5% to 95.5% where as in Summer it was completed in 528 hrs (22 days), when the temperature ranged from 26.5°C to 35.1°C and the humidity ranged from 34% to 67.5%. The P value was found to be  $P < < 0.05$  in Temperature variation; and  $P < < 0.05$  in Humidity variation in different stages(1st instar larvae, 2nd instar larvae, 3rd instar larvae, Prepupae, Pupae and Adult).

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**Submission date:** Sep 2, 2024

**Revision date:** Oct 7, 2024

**Published date:** October 28, 2024

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**Conclusion:** In the present study we have found that fluctuating temperature and relative humidity of outer condition in two seasons, Rainy and Summer effect the life cycle duration of the fly from the larvi position up to adult eclosion and statistical analysis has proved it to significant rejecting null hypothesis. This data can be further used in the estimation of Post Mortem Interval (PMI) in Homicide and Suspicious cases of both human and wild life poaching death cases.

**Key words:** Forensic entomology, *Sarcophaga dux* (Thomson ,1869), Temperature, Relative Humidity, Larvae, P value

## Introduction

The presence of some species of insects, including their immature stages can provide information about the location, time and condition of cadavers and hence forensic entomologists make use of the same in crime investigations as reported by ( Bharti et.al. 2007<sup>1</sup>; Byrd and Castner 2010<sup>2</sup>; Greenberg1991<sup>3</sup>; Goff 2000<sup>4</sup>; Singh and Bharti 1999<sup>5</sup>; Suri Babu 2008<sup>6</sup>). The blow flies are the most important forensic indicators because they are usually the first to colonize the carcass, often within minutes or even seconds of exposure, the flies were *Calliphoravicina* at constant temperature of 10,12.5,19,22.5°C, *M. scalaris* at 22 and 29°C by Greenberg 1991<sup>3</sup> while impact of place where crime has occurred has a varying temperature which hugely impacts the growth of fly development which is taken in to consideration in this study. The flesh flies belong to the family Sarcophagidae, currently in the world there are 133 genus and 3079 species as reported by Roskov Y et.al 2016<sup>7</sup>.

The insects most frequently used to estimate PMI and as toxicological analysis samples belong to the families Calliphoridae (blowflies) and Sarcophagidae (flesh flies). These flies are expert agile flyers that feed on corpses, and are usually the first to arrive to the corpse or death scenario as reported by Goff et. al. 1994<sup>8</sup>. Family Sarcophagidae have been found both in Britain and elsewhere, to be present on the body after a few days of decomposition. One of the most significant things about this family is that the flies are viviparous, i.e. they generally lay larvae on the corpse and not lay eggs as report by Gennard DE 2007<sup>9</sup>.

The first insects to arrive under most circumstances are the Calliphoridae (blow flies) and Sarcophagidae (flesh flies). Female flies will arrive and begin to explore the potential sites for oviposition or larviposition. Forensic entomologists may have difficulties in estimating PMI when species that are hard to identify are collected, such as Sarcophagidae

and Phoridae (3,000 species in the world). The main consequence in this situation is also the lack of development data of some species in these families as reported by Amendt et. al. 2010<sup>10</sup>. The Indian family of Sarcophagidae consists of 126 species, out of which 89 species are from the genus *Sarcophaga* which amounts for 70% of the Indian Sarcophagidae family as reported by Chakraborty et.al. 2017<sup>14</sup>.

Sarcophagidae are considered to be unimpeded by rain and to fly despite the weather as reported by Erzinclioglu, 2000<sup>16</sup>. In recent times in India Abd-Algalil et. al. 2017<sup>19</sup> have studied on *Chrysomyasaffraneae* (Bigot, 1877), effects of variation in temperature and humidity on the different life cycle stages of Calliphoridae fly *Chrysomyasaffraneae* (Bigot, 1877), but it has been conducted at laboratory condition, as per usual crime scenes, dead bodies are mainly found in the open sites. So climatic condition with fluctuating temperature and humidity plays an important role as reported in our previous paper, Suri Babu et. al. 2013<sup>15</sup>. We have formulated a pioneer first method in South East Asia for determining post mortem interval (PMI) of dead body lying in open for over 4 days with inclusion of temperature of the given territory of that area of given time period and we developed formulation which gave accurate time of death, in same manner we have reared flies in open taking in account of temperature and relative humidity of the area. We have tried to find out changes in stages of rearing and variation in timing of rearing stages by the impact of temperature and relative humidity of that area, whether significant variation is found in seasonal variation or not.

## Material and Methods

The present study was carried out in the outer premises of the Regional Forensic Science Laboratory Building, Jagdalpur, Bastar dist, Chhattishgarh, India. A detailed study of the pattern of succession of flesh fly was done in Rainy season July 2012 and Summer season June 2012.

The fresh mass of meat of malegoat was transferred to a 500ml plastic container in which mixture of saw dust and soil was placed at the bottom. The mouth of the container was closed with a perforated lid to facilitate air circulation and to prevent newly emerged flies escaping from the container. A Sket© brand Germany made Maxima-Minima thermometer was used for recording of maximum and minimum daily temperature which were later tallied with local weather station data.

The maggots were observed daily and different immature stages and adults were collected and identified with the help of taxonomic keys of White

RS, 1923<sup>11</sup>, White RS et.al. 1940<sup>12</sup>, Smith KGV 1986<sup>13</sup>, Chakraborty et.al.2017<sup>14</sup>, Amendt et. al. 2010<sup>10</sup> and further identification wasconformed by the Zoological Survey of India, Kolkata, West Bengal, India.

The flies were identified to be of Order: Diptera; Family: Sarcophagidae; Species: Sarcophaga (Liosarcophaga) dux (Thomson,1869).

The Meteorological data: temperature, rainfall and relative humidity were obtained from The Government Agromet Observatory, Shaheed Gundadhoor College of Agriculture and Research Station, Kumaravand, Jagdalpur-494001,CG,India.

**Growth pattern of *Sarcophagadux* reared in season of Rainy and Summer season:**

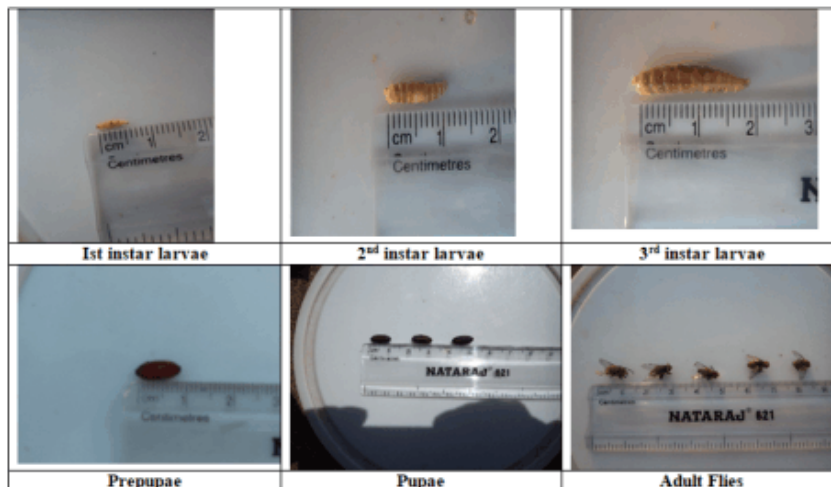
**Table 1: Growth pattern of Rearing of *Sarcophaga dux* in Rainy season.**

Stage	Length (mm)	Time Spent in Stage(Days)
1 <sup>st</sup> Instar Larvae	5	3 (19/07/12-21/07/12)
2 <sup>nd</sup> Instar Larvae	11	1 (22/07/12)
3 <sup>rd</sup> Instar Larvae	19	1 (23/07/12)
PrePupae	8	1 (24/07/12)
Pupae(P)- Adult emergence(A)	10(P)- 12(A)	5 (25/07/12-29/07/12)

**Table 2: Growth pattern of Rearing of *Sarcophaga dux* inSummer season.**

Stage	Length (mm)	Time Spent in Stage(Days)
1 <sup>st</sup> Instar Larvae	5	2 (02/06/2012-03/06/12)
2 <sup>nd</sup> Instar Larvae	11	1 (04/06/12)
3 <sup>rd</sup> Instar Larvae	19	3 (05/06/12-07/06/12)
PrePupae	8	3 (08/06/12-10/06/12)
Pupae(P)- Adult emergence(A)	10(P)-12(A)	13 (11/06/12-23/06/12)

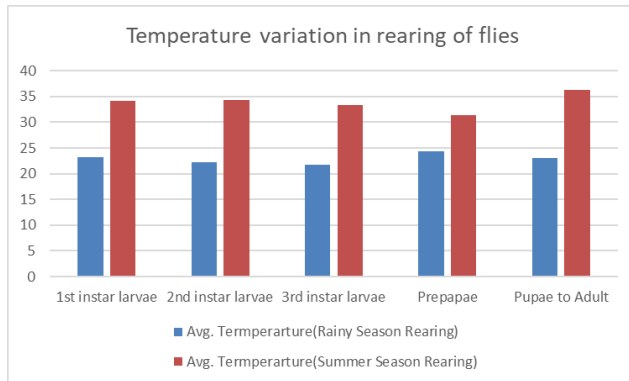
**Table 3: Photographs of different stage of life cycle of *Sarcophaga dux*: 1<sup>st</sup> instar larvae, 2<sup>nd</sup> instar larvae, 3<sup>rd</sup> instar larvae, Prepupae, Pupae, Adult.**



**Table 4: Meteorological data of 18/07/12-29/07/12 (Rainy season) and 01/06/12-23/06/12 (Summer season) for Average Temperature, Rainfall and Average Relative humidity.**

S. No	Meteorological data of 18 <sup>th</sup> July, 2012-29 <sup>th</sup> July 2012(Rainy)				Meteorological data of 01 <sup>st</sup> June, 2012-23 <sup>th</sup> June 2012(Summer)			
	Date	Avg Temperature	Rainfall	Avg. Humidity	Date	Avg Temperature	Rainfall	Avg Humidity
1	18/07/12	24.6°C	0.0	87%	01/06/12	34°C	0.0	38.5%
2	19/07/12	24.5°C	18.2	91%	02/06/12	33°C	0.0	33%
3	20/07/12	22.5°C	18.8	93.5%	03/06/12	35.1°C	0.0	42.5%
4	21/07/12	22.6°C	20.0	92%	04/06/12	34.3°C	0.0	34%
5	22/07/12	22.3°C	40.2	95.5%	05/06/12	33.9°C	0.0	31.5%
6	23/07/12	21.8°C	17.4	79.5%	06/06/12	34°C	0.0	40.5%
7	24/07/12	24.3°C	1.2	83.5%	07/06/12	32°C	0.0	42%
8	25/07/12	23.3°C	35.2	88%	08/06/12	32.1°C	0.0	35.5%
9	26/07/12	23.6°C	15.8	93%	09/06/12	32.3°C	0.0	41.5%
10	27/07/12	22.3°C	8.0	88.5%	10/06/12	29.8°C	47.4	72.5%
11	28/07/12	22.4°C	4.4	80%	11/06/12	25.8°C	0.0	66.5%
12	29/07/12	24°C	6.8	82.5%	12/06/12	28.8°C	0.0	54%
13					13/06/12	28°C	5.2	56.5%
14					14/06/12	29.3°C	0.0	54.5%
15					15/06/12	29.8°C	0.0	50.5%
16					16/06/12	30.9°C	0.0	54.5%
17					17/06/12	27.8°C	5.2	75%
18					18/06/12	24.8°C	2.0	70%
19					19/06/12	24.95°C	0.0	69.5%
20					20/06/12	25°C	3.2	79.5%
21					21/06/12	24.1°C	7.6	84.5%
22					22/06/12	22.5°C	15.8	89.5%
23					23/06/12	23.3°C	2.8	73.5%

**Statistical analysis for Temperature variation in *Sarcophaga dux* rearing in weather condition of Rainy and Summer season:**



**Figure 1: Temperature variation in stages of rearing of *Sarcophaga dux* (Thomson, 1869) in weather condition of Rainy and Summer Season.**

Mean of temperature variation in Rainy and Summer season =  $\sum X_{msrg} / X_{msrg} = 22.9$  (Rainy season),  $33.9$  (Summer season)

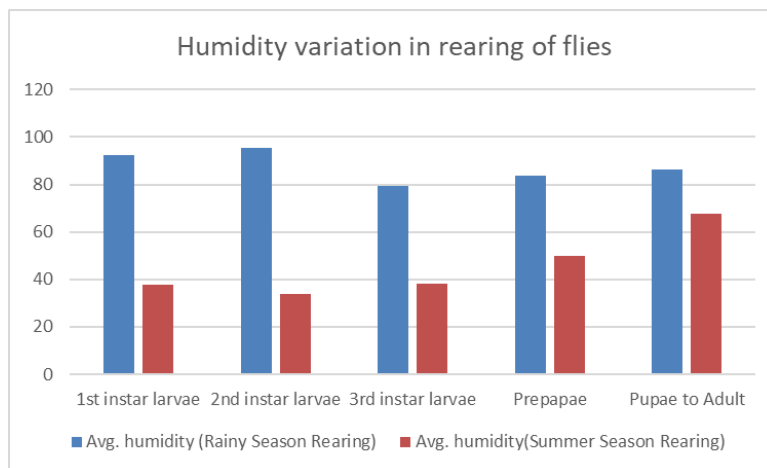
Overall mean:  $\bar{X} = 28.4$

$X_{ts}$  = Average temperature at different stages (1<sup>st</sup> instar, 2<sup>nd</sup>, 3<sup>rd</sup> instar, prepupae, P-A) respectively of growth period,  $t$  = temperature and  $s$  = stage No. of observations in average temperature in stages (n) = 5, Sets of data taken (a) = 2, Number of stages data (N) = 10

**Table 5: Hypothesis testing for Temperature variation in different stages *Sarcophaga dux* (Thomson, 1869) rearing in weather condition of Rainy and Summer season.**

$SS_B$ (between Rainy and Summer season) = $n \sum (X_{msrg} - \bar{X})^2 = 302.5$	$df_B = a - 1 = 1$	$MS_B = SS_B / df_B = 302.5$	$F_{stat} = MS_B / MS_E = 149.8$  $F_{critical} = F_{(df_B, df_E)} = 5.32$	
$SS_E$ (with in Rainy and Summer season/error within) = $\sum (X_{ts} - X_{msrg})^2$ With in group variation = 16.23	$df_E = N - a = 8$	$MS_E = SS_E / df_E = 2.02$	So, $F_{stat} > F_{critical}$  Hence, Reject null hypothesis, significant difference in temperature variation in different stages of rearing of <i>Sarcophaga dux</i> in Rainy and Summer season	The P value = 0.00000195, is less than 0.05

**Statistical analysis for Humidity variation in *Sarcophaga dux* rearing in weather condition of Rainy and Summer season:**



**Figure 2: Humidity variation in stages of rearing of *Sarcophaga dux* in weather condition of Rainy and Summer Season.**

Mean of humidity variation in Rainy and Summer season =  $\bar{X}_{msrg} = 87.4$  (Rainy season),  $\bar{X}_{msrg} = 45.4$  (Summer season).

Overall mean:  $\bar{X}_{..} = 66.4$ ,

$X_{ts}$  = Average humidity at different stages (1<sup>st</sup>

instar, 2<sup>nd</sup>, 3<sup>rd</sup> instar, prepupae, P-A) respectively of growth period,  $t$  = temperature and  $s$  = stage.

No. of observations in avg. humidity in stages (n) = 5, Sets of data taken (a) = 2, Number of stages data (N) = 10

**Table 6: Hypothesis testing of humidity variation in different stages *Sarcophaga dux* rearing in weather condition of Rainy and Summer season.**

$SS_B$ (between Rainy and Summer season) = $n - 1)^2 = 4411.9$	$df_B = a - 1 = 1$	$MS_B = SS_B / df_B = 4411.9$	$F_{stat} = MS_B / MS_E = 38.6$ $F_{critical} = F_{(dfB, dfE)} = 5.32$	
$SS_E$ (with in Rainy and Summer season / error within) = With in group variation = 913.5	$df_E = N - a = 8$	$MS_E = SS_E / df_E = 114.2$	So, $F_{stat} > F_{critical}$  Hence, Reject null hypothesis, significant difference in humidity variation indifferent stages of rearing of <i>Sarcophaga dux</i> in Rainy and Summer season	The P value = 0.000259, is less than 0.05

**Result**

In the variation of temperature of Rainy and Summer season:-The P value was found to be 0.00000195, and  $P < 0.05$ . In the variation of relative humidity of Rainy and Summer season:-P value

was found to be 0.000259, and  $P < 0.05$  indifferent stages (1<sup>st</sup> instar larvae, 2<sup>nd</sup> instar larvae, 3<sup>rd</sup> instar larvae, Prepupae, Pupae-Adult) of *Sarcophaga dux* proving there is a significant difference in of Temperature and Humidity variation in stages of rearing in different seasonal conditions.

## Discussion

Mahmood et. al. 2012<sup>17</sup> have reported abundance of pre-pupae of Calliphorid and Sarcophagid flies preferring a particular pupation was observed against six different pupation substrates. These substrates were commonly found in the vicinity of human habitats. This experiment was designed to trace the forensically important pre-pupae with reasonable accuracy. Variations among the all six pupation substrates were differing significant ( $p < 0.001$ ). Significant variations were observed between the batches ( $p < 0.01$ ). In our study also Temperature variation and Relative humidity variation in *Sarcophaga dux* rearing in weather condition of Rainy and Summer season, Variation was found to be differing significant to be  $P = 0.00000195$  ( $P < < 0.05$ ) in Temperature variation and  $P = 0.000259$  ( $P < < 0.05$ ) in humidity variation in different stages of fly development.

Grassberger et. al. 2002<sup>18</sup> have reported development time from oviposition to adult eclosion was shortest at 35.8°C and longest at 15.8°C. In our study we have found the development time from 1st instar larvae to adult fly was shortest at 34.3°C with 2nd instar larvae of *Sarcophaga dux* stage taking 24 hrs in Summer season rearing of fly and longest at 36.3°C with pupae to adult stage of *Sarcophaga dux* stage taking 312 hrs in Summer season rearing of fly.

Abd-Algalil et. al. 2017<sup>19</sup> have reported the total life cycle duration of *Chrysomya affranaea* (Bigot, 1877) in Summer was completed in 220 hrs (9.17 days) when the temperature ranged from 30.5°C to 33.2°C and the humidity ranged from 12% to 19%, but in Rainy season it was completed in 259 h (10.79 days) when the temperature ranged from 25.6°C to 28.9°C and the humidity ranged from 50% to 65%. and the shortest life cycle duration was recorded in Summer, then followed by the Rainy season. While in our study the total life cycle duration of *Sarcophaga dux* in Summer was completed in 528 hrs (22 days), when the temperature ranged from 26.5°C to 35.1°C and the humidity ranged from 34% to 67.5%, but in Rainy season it was completed in 264 h (11 days) when the temperature ranged from 21.8°C to 24.5°C and the humidity ranged from 79.5% to 95.5%. The shortest life cycle duration was recorded in Rainy,

then followed by the Summer season.

Abd Algalil et. al 2015<sup>20</sup> have reported that in their study on the developmental duration of *Chrysomya megacephala* (Diptera: Calliphoridae) the total life cycle duration in Rainy season was completed in 265 h  $\pm$  2 h (11.04 days  $\pm$  0.08 days) when the temperature ranged between 26°C and 29°C and humidity ranged between 35% and 50%. While in our study the total life cycle duration of *Sarcophaga dux* in Rainy season was completed in 124 h  $\pm$  4 h (11.05 days  $\pm$  0.06 days) when the temperature ranged between 21.8°C and 24.3°C and humidity ranged between 79.5% and 95.5%.

Warren et. al. 2013<sup>21</sup> compared the immature development of (Diptera: Calliphoridae) *Protophormiaterraenovae* (Robineau-Desvoidy, 1830) at fluctuating temperatures of 4-28 and 9°C to 23°C and at their mean constant temperature 16°C, they reported that the development was fastest at the greater fluctuation and slowest at the constant temperature and showed similar percentages of development time in each stage. They conclude that the fluctuations above the mean enhance the developmental rate relatively more than temperatures below the mean can reduce the developmental rate. In our study in Rainy season, development of *Sarcophaga dux* at fluctuating temperature of 21.8-24.5°C and mean temperature of 22.9°C and in Summer season, development at fluctuating temperature of 31.4°C -34.3°C and mean temperature of 33.9°C, The development was fastest in heavy fluctuation of temperature in Rainy season and slowest in less fluctuating temperature in Summer season.

## Conclusion

From present study we can conclude that shortest life cycle duration of *Sarcophaga dux* was in Rainy season with moderate temperature and high relative humidity where as in Summer season longest life cycle duration was observed where high temperature and low humidity was noticed. The value of  $P < < 0.05$  was found Temperature variation;  $P < < 0.05$  was found in Humidity variation in different stages (1<sup>st</sup> instar larvae, 2<sup>nd</sup> instar larvae, 3<sup>rd</sup> instar larvae, Prepupae, Pupae-Adult) of this fly proved significant difference lies in variation and hence it rejects null hypothesis proving Temperature and

Relative Humidity do cause significant variation in different stages of fly development, proving vital for the cases of Homicide and Suspicious death cases of both human and wild life poaching indetermination of Post Mortem Interval(PMI) as previously theKulshrestha et. al 2005<sup>22</sup> the rearing of the flies in the actual environmental of crime scene place was not considered, in our study we have statistically proved that environmental variation does play a significant role in the growth pattern of forensic flies, this area of variation in environment need to be expanded as in India there is huge variation in climatic condition in different places a further broad level study needs to be done in this field as it implies a huge factor in determination of Post Mortem Interval in highly decomposed bodies where traditional parameters of determining times since death are not applicable.

**Acknowledgements:** The authors are deeply indebted to:

Chhattisgarh, Bastar region, India: Mr. Sundereraj.P, IPS, Inspector General of Police, Bastar Range(CG) , Mr.Salabh Kumar Sinha, IPS, Senior Supdt of Police, Bastar District, CG.

Andaman & Nicobar Islands, India: Mr. Hargobinder Singh Dhaliwal, IPS, Director General of Police A&N islands; Mr. Rajeev Ranjan, IPS SP(CID); Mrs. Varsha Sharma, IPS, DIGP(L&O,PMF);Mr. Kumar Abhishek, Dy SP/DDO(CID);Mr. Vijay Singh, Dy SP, SDPO Car Nicobar and SHONancowry Mr Calvin for support and encouragement for Forensic Entomological studies.

And thankful to Mr. J.L. Choudhary, Jagdalpur (C.G) for providing meteorological data.

**Ethical Clearance:** since the subjects were flies, ethical clearance is not required

**Source of Funding:** Self

**Conflict of Interest:** Nil

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