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EFFECT OF VARIED TEMPERATURE CONDITIONS ON THE DEVELOPMENT OF A CALLIPHORID FLY *CHRYSOMYA RUFIFACIES* (MACQUART 1843) OF OSMANABAD DISTRICT (MH), INDIA.

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Abstract:

In forensic entomology insect evidences and the developmental stages of the insects used to determine the post-mortem interval (PMI). A PMI estimation is based either on insect developmental rates or on insect colonization and succession patterns of carrion. Insect activity is highly influenced by temperature, which can vary, based on season and geographic location. Carrion decomposition studies conducted in various geographic locations and among seasons within one geographic location is therefore a necessity in developing a baseline data set for use in the field of forensic entomology. *C. rufifacies* (Macquart 1843) is one of the Calliphorid flies with forensic and medical importance has been studied for the effect of seasonal variations on its life cycle duration and morphological parameters. Results shows that in summer season life cycle of the fly is completed in 218 hrs. In rainy and winter season life cycle is completed in 277 hrs and 317 hrs. Respectively. High temperature in summer resulted in completion of life cycle within a short duration and size of the maggots were small at each stage as compared to winter and rainy season. Length, width and weight of the maggots recorded more in rainy season and life cycle duration was prolonged. The results of the present study shows that warmer temperatures during summer and rainy seasons speeded up the succession by accelerating the development and activity of the maggots. Whereas, cooler temperatures of winters retarded the development and activity of the maggots. This affords an excellent opportunity to make a significant contribution to the baseline database used in forensic entomology.

Key words: *C. rufifacies*, temperature, humidity, entomology, developmental, cadavers, succession.

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Introduction:

Forensic entomology is the branch of forensic science in which information about insects are used to draw conclusions while investigating legal cases relating to both humans and wildlife, although on occasion the term may be expanded to include other arthropods. Insects used in the investigation of a crime scene both on land and in water (Anderson, 1995; Erzinclioglu, 2000; Keiper and Casamatta, 2001; Hobischak and Anderson, 2002; Oliveira-costa and De Mello-Patiu, 2004).

Forensic entomology uses the insect evidences to estimate the developmental time of insect. It also observes the carrion insect succession to estimate time since death. As death occurs, using a variable amount of time prior to colonization the entomologists estimate the minimum PMI (m PMI). By estimating the earliest period, the body colonized by insects to the time for the collected specimens for a certain developmental stage (Villet *et al.*, 2010). The maximum PMI (max PMI) calculated from when the person last seen alive to the discovery of the body (Villet *et al.*, 2010).

Forensic entomology uses insects to help law enforcement to determine the cause, location and time of death of a human being. Insect life cycles act as a precise clock, which begin within minutes of death. It used to determine the time of death when other methods are useless (Catts and Haskell, 1990).

Entomological tools used to determine if the body moved from one locality to another and may provide information about the site of death. It also defines diversity of insects if the body has moved from one locality to another and may provide information about the site of death itself because of the relatively defined diversity of insets that exist in specific geographical area or habitat (Anderson, 2009).

In the first 72 hours after death, the pathologist considered able to provide a reasonably accurate determination of the time of death. This based upon the condition of the body itself and such features as the fall in body temperature. Beyond this time, there is less medical information with which to correlate PMI. So another area of expertise is required to clarify the time of death. The forensic entomologist can provide a measure of the possible post mortem interval, based upon the life cycle stages of particularly fly species recovered

from the corpse or from the succession of insects present on the body. This estimate will give over a period of hours, weeks or years. The start of the post mortem interval calculated to coincide with the point when the fly first laid its eggs on the body and its end to be the discovery of the body and the recognition of life stages of the oldest colonizing species infesting it.

The duration of the developmental stage, in relation to the particular stage of decay, gives an accurate measure of the probable length of time the person has been dead and may be the best estimate that is available (Gennard, 2007). Dipteran families namely Callipohoridae, Sarcophagidae, Muscidae, Sepsidae, Sphaeroceridae, Piophilidae, Phoridae and Coleopteran families namely Histeridae, Styphylinidae, Siliphidae, Cleridae, Dermestidae, Tenebrionidae predominate the scene (Reed, 1958; Payne, 1965; Braack, 1986; Goff et al., 1986; Goff 1993; Tantawi et al., 1996). These insects form a complex food web within the carrion. Taxa actually feeding on the corpse includes many of the true flies (Diptera) particularly the blowflies Calliphoridae and flesh flies Sarcophagidae who are early invaders and Beetles (Coleoptera, Siliphidae and Dermestidae). This group includes species that may be the most significant isolatable taxa for use in estimating a minimum period of insects activity on the body during the early stages of decomposition i.e. days 1 to 14 (Amendt et al., 2009). Out of all the insects visiting a dead body. The maggots of blowflies calliphoridae and flesh flies sarcophagidae are responsible for the maximum consumption of terrestrial carrion (Fuller, 1934; Payne, 1965; Putman, 1977; Putman, 1978; Braack, 1981; Early and Goff, 1986).

Calliphoridae :

Members of this family usually known as blowflies, blue bottles or green bottles due to their blue, green, yellow, brown, grey and black colour. They are generally oviparous (a few are viviparous) and breed in decaying animal matter and on living animals. The adults of this family can distinguished from other related families in having well developed thoracic squamae, two notopleural and a distinct row of hypopleural bristles and the larvae in having posterior spiracles in slight in slight depression in posterior plate (Nandi, 2002). The Calliphoridae are of great importance in the field of forensic entomology as these flies are attracted to decomposing remains and knowledge of the timing and duration of

their life cycle has been widely used to underpin time since death calculations. The Calliphorids exhibit the ability to exploit nutrient rich sources such as carrion, domestic and commercial food waste, making them important in the decomposition process (Zumpt and Patterson, 1952; Norris, 1965; Smith, 1986). Primarily the eggs of calliphoridae be found in sites that provide good conditions for position, mainly moist areas including orifices, open wounds and underneath clothing that prevent the desiccation of eggs (Erzinclioglu, 1986). In India, calliphoridae represented by 119 species belonging to 30 genera under 09 subfamilies (Bharti, 2011; Mitra and Sharma, 2013).

Materials and Methods:

Flies of calliphoridae and sarcophagidae were collected in different seasons from the different places of Osmanabad district of Maharashtra state (India) during 2013 to 2016. Osmanabad is one of the major district of Marathwada regions of the Maharashtra. It situated in the southern part of the state abutting Andhra Pradesh in south and lies in between North latitudes 17037' and 18042' and least longitude 75016' and 76047' and falls in parts of survey of India degree sheets 47N, 47O, 58B and 56C. The geographical area of the district is 7512 sq. km.

The laboratory rearing of forensically important insects can provide the forensic entomologists with an invaluable database of insect development under controlled environmental conditions. It may play a very important role in determining PMI. Sometimes it is very difficult to rear some insect species within a laboratory environment. It can prove to be an extremely difficult task, but fortunately, the needs of most insects of forensic importance can easily met in captivity.

For collection, the fresh liver sample purchased from the local slaughterhouse. Partially putrefied liver/meat exposed in the air and within few minutes, the flies attracted. Similarly, maggots and adult flies collected from dead bodies of roadkill cadaver from different regions of Osmanabad district. Insects collected for culturing brought to the laboratory and kept in conditions, which enable them to grow successfully. Adults fed daily on the fresh beef liver and water mixed with honey. The fresh liver used daily as an oviposition site for females as it is a rich source of protein. Hygiene and cleanliness maintained in the

rearing boxes to avoid any infection to the culture.

Results and Observation:

Life cycle of *C. rufifacies* in summer

To study the seasonal effects on the life cycle of *C. rufifacies* in summer five replicates taken and their average considered. Results of the developmental stages of *C. rufifacies* in summer are as follows.

Eggs:

The development of *C. rufifacies* is holometabolous which is also called as complete metamorphosis like other calliphorid flies. The average length, width and weight of the eggs of *C. rufifacies* were 1.4 mm, 0.6 mm and 0.51 mg respectively. Recorded temperature, humidity at this developmental stage was 34.8^{0}C and 30%. The eggs hatched after 23 hrs into 1st instar larvae.

Larvae (Maggot):

The life cycle of *C. rufifacies* in summer showed that first instar larva emerged from the egg after 23 hrs of development and remained in this stage for 27 hrs up to 50 hrs. The average length of larvae at this developmental stage was 4.7 mm, width 1.2 mm and weight 8.2 mg. Recorded temperature, humidity was 34.4^{0}C and 26 %.After 50 hrs of development the first instar larvae molted into second instar larvae, which remained in this stage for 36 hrs up to 86 hrs. The larvae observed as a voracious feeder at this stage of development and they grew very fast. The average length of second instar larvae was 8.9 mm, width 2.3 mm and 20.3 mg. Recorded temperature, humidity was 32.6^{0}C and 28%. The average length of third instar larvae at this developmental stage was 14 mm, width 3.5 mm and weight 51.2 mg.

Prepupae:

During pupation the colour of prepupae changed from white creamy to dark brownish colour, this stage called as pupae. This is a non-feeding stage when maggots stop feeding and undergo the resting stage. At this time, the prepupae start searching for the dry and safe place for pupation. Prepupa stage remained from 86 to 116 hrs and the size of the prepupa shrunk. The length of prepupae was 12.1 mm, width 3.8 mm and weight 42.8 mg.

Pupae:

After the prepupae transformed into the pupae, the pupae became darker

brownish in colour. Pupal developmental stage was remained from 116 to 218 hrs during the development indicating, the time spent in the pupal stage as 102 hrs. The average length and width remain constant at this stage because of the rough puparia but the weight starts decreasing slightly until the adults start emerging out from the pupae.

Adult:

After 218 hrs of development (09 days, 2 hrs.) adults emerged out from pupae. Recorded temperature, humidity at this stage was 31.6^{0}C and 26 %. The life cycle of *C. rufifacies* flies in summer completed in 218 hrs. The adult's length, width, and weight were 8 mm, 3.5 mm and 30 mg respectively. The details of the PMI and the effects of temperature and relative humidity and morphological parameters in summer and variations during the development given in the table: no.1.

Life cycle of *C. rufifacies* in Rainy season

To study the seasonal effects on the life cycle of *C. rufifacies* in rainy season five replicates taken and their average considered. Results of the developmental stages of *C. rufifacies* in summer rainy are as follows.

Eggs:

The average length, width, and weight of the egg were 1.2 mm, 0.4 mm and 0.47 mg respectively. The development of the eggs to first instar larvae completed after 23 hrs and the recorded temperature, humidity was 29.1^{0}C and 76%.

Larvae (Maggots):

The first instar larvae emerged out from eggs after 23 hrs of development. Recorded temperature and humidity was 29.6^{0}C and 74%. The first instar larvae developed up to 47 hrs the average length of first instar larvae was 4.2 mm, width 1 mm and weight 7.4 mg respectively. The first instar larvae grew further for 24 hrs and molted into 2nd instar larvae when 29.7^{0}C was the temperature and 72% humidity was recorded. It remained in this stage from 24 hrs up to 88 hrs the average length of 2nd instar larvae was 7.8 mm, width 1.8 mm and weight 18.3 mg. The second instar larvae molted to the third instar larvae after 88 hrs of development. Recorded temperature, humidity at this developmental stage was 27.5^{0}C and 72 %.The time spent in third instar larval stage was 73 hrs from 88 to 161 hrs. The average length of the third instar

was 13.4mm,width 2.7 mm, and weight 40.2 mg respectively.

Prepupae:

The third instar larvae attained their full growth at this developmental stage, stopped feeding and started searching for a dry and safe place for pupation. Prepupa was very sensitive for the wetness. These non-feeding larvae leave the food or decay meat and start to burrow deep into the soil. The duration of this stage ranged from 161 to 277 hrs. The average length of the prepupa was 11.6 mm, width 3.5 mm, and weight 40.8 mg. At the end of this stage, larva began to transform into pupae.

Pupae:

During this stage the prepupae becomes darker from white to light brown and dark brownish and became shorter, pupa stage remains from 161 to 277 hrs during the development. The average length of the pupa was 8.8 mm, width 3.4 mm, and weight 34 mg.

Adults:

Development of the fly completed in 277 hrs. Adults emerged from puparia after 277 hrs when 29.2 was the recorded temperature and 67% humidity. The duration of the live cycle of *C. rufifacies* flies completed in 277 hrs (11 days, 13 hrs). The average length of the adult was 8.2mm width 3.3 mm, and weight 27 mg. The PMI and the effect of temperature variation and relative humidity recorded during the experiment shown in table no. 2.

Life cycle of *C. rufifacies* in winter season

To study the seasonal effects on the life cycle of *C. rufifacies* in winter season five replicates taken and their average considered. Results of the developmental stages of *C. rufifacies* in winter are as follows.

Eggs:

Eggs had length 1.6 mm, width 0.7 mm and weight 0.54 mg. The egg required 24 hrs for hatching.

Larvae (Maggot):

Development of the eggs to 1st instar larvae was completed in 24 hrs on 24th hrs of the development 1st instar, larvae emerged out from the egg and remained for 25 hrs in 1st instar. The average length of the first instar larvae was 5.3 mm, width 1.7 mm and weight 9.8 mg. Recorded temperature, humidity was 26.8°C and 60%. When recorded temperature, humidity was 26.7°C and 48%. After 49 hrs 1st instar larva molted into 2nd instar larvae when the average

length of second instar larvae was 9.5mm,width 3 mm and weight 25.2 mg. on the 91st hrs of the development,the 2nd instar larvae molted into 3rd instar larvae which fed up to 139 hours from the egg laying. Recorded temperature, humidity at this stage was 17.2^{0}C and 52.5%. The average length, width and weight of 3rd instar larvae were 16 mm, 4.2 mm and 58.5 mg respectively.

Prepupae:

At this developmental stage the maggots stopped feeding and undergo to the resting stage, the prepupae searched for the dry and safe place for the pupation. The duration of this non-feeding stage is 48 hrs started from 91 to 139 hrs from the egg laying. The average length of prepupae was 13.3 mm, width 4 mm and weight 45.6 mg.

Pupae:

During the stage prepupae transformed into the pupae. During the puparia of the prepupae darkened from white to dark brownish in colour. Pupal stage remained for 178 hrs. The average length, width and weight of pupae were 9.2 mm, 3.8 mm and 38.7 mg respectively. The pupa wasinitially light brown in colour, which later became dark brown as the development continues.

Adult:

Development up to the adult completed in 317 hrs. Adult emerged out from pupae on the 317 hrs of development when the recorded temperature, humidity was 24.1^{0}C and 39% respectively. The life cycle of *C. rufifacies* flies in winter completed in 317 hrs (13 days, 5 hrs.). Adult length, width, and weight were 8.5 mm, 3.6 mm and 33.1 mg respectively. The PMI and the effect of temperatures variation and relative humidity recorded shown in Table: no.3. PMI in hours of different developmental stages of *C. rufifacies* in different seasons has shown Figure: 5.1. The morphological parameters of different developmental stages of *C. rufifacies* in different seasons shown in Figures: 6.1 to 6.3. Figures 1, 2, 3, 4 shows graphical representation of effect of temperature and humidity on development and morphological parameters of *C. rufifacies* in different season.

Discussion:

Insect's developmental rate and succession pattern on the carrion differ from country to country and even from area to area within the different parts of the same country. Main reason behind this variation is

the topography and environmental conditions. Therefore, it is impossible to apply the data available in one country and to any other country in the field of forensic entomology. A collection of information of insect's developmental data as per region is mandatory for using insect evidence in criminal investigations.

This research is important in the field of forensic entomology. As it is the application of arthropod science in the judicial system. Forensic entomologist assist in criminal cases by estimating the time of insect colonization of human or other animals (Keh, 1985). Forensic entomologists mainly rely on laboratory developmental data in order to make these estimates. Colonization of the arthropods occurs soon after the death of an animal. These estimates are synonymous with the m PMI. The need for development data is necessary, as they might be significantly different from each species as well as various environmental conditions (Tarone and Foran, 2006, Boatright and Tomberlin, 2010).

A postmortem interval estimation (PMI) based on insect developmental rates or on insect colonization and succession patterns at carrion (Catts and Goff, 1992), and insect activity is highly influenced by temperature, which canvary, based on season and geographic location (Introna *et al.*, 1991; Haskell*et al.*, 2001). In general, climatic conditions particular temperature, play an important role in the insect activity and carrion decomposition. Variations in climatic conditions lead to differences in the decomposition speed, insect developmental rate and succession pattern in different habitats, seasons and geographic locations (Anderson 2009).

Mann *et al.*, (1990) have ranked temperature as the number one variable affecting decomposition rates of human cadavers in Tennessee and found that during the winter months with the cold and freezing temperatures, the decomposition process proceeds at a greatly reduced rate. Carvalho and Linhares (2001) studied pig carcass decomposition in Brazil. They examined seasonal variation in the duration of the decomposition process and found that as the average temperature decreased for each season, the duration of the decomposition process increased. They found the reverse to be a time in the spring and summer. The results of the studies discussed above are consistent with the result of this study.

Low temperatures (De Jong and Chadwick, 1990; Shah and Sakhawat, 2004),

heavy rain, and humidity (Smith, 1986) influence blowfly activity and delay their arrival at carrion. Greenberg (1990) has stated that Calliphorids do not fly in the rain. Similarly, Digby, (1958) found that strong wind inhibited the ability of *Calliphora vicina* to fly. In contrast, Sarcophagid flies considered to be unimpeded by rain (Erzincliozlu, 2000); as a result, flesh flies may be the initial colonizers of the body if there is a long period of rainy weather. Temperatures above 30^0C and below 12^0C known to inhibit blowfly activity (Gennard, 2007).

PMI estimation based on insect developmental rates of insect colonization and succession patterns at carrion (Catts and Goff, 1992) and insect activity is highly influenced by temperature, which can vary, based on season and geographic location (Introna *et al.*, 1991; Haskell *et al.*, 2001). Carrion decomposition studies conducted in various geographic locations and during varying seasons within one geographic location is therefore a necessity in developing baseline data for use in the field of forensic entomology.

The eggs of *C. rufifacies* hatched after 18 hours in summer when the average temperature and relative humidity were 33.8^0C and 30%. In the rainy season hatched after 22 hrs when the average temperature and relative humidities were 27.45^0C and 58%, while in winter hatched 28 hrs when the average temperature and relative humidities were 29.1^0C and 76%.

The first instar larvae of *C. rufifacies* in summer remained for 23 hrs when the average temperature and relative humiditiy were 34.4 ^{0}C and 26 %. In a rainy season it spent 23 hrs when the average temperatures was 29.6^0C and average relative humidity was 74 %, while in winter it spent 24hrs when the average temperature and relative humidity was 26.8 ^{0}C and 60% respectively. The sizes of the first instar larvae in summer were bigger than the sizes in winter and smaller than the sizes in the rainy season.

The second instar larvae developed in 27 hrs in summer when the average temperature and relative humidity were 34.1 ^{0}C and 27 %. It spent 24 hrs in the rainy season when the average temperature and relative humidities were 29.7 ^{0}C and 72 %. In winter when the average temperature and relative humidity were 26.7 °C and 48 % 2nd instar larvae spent 25 hrs The sizes were nearly doubled because all instar larvae were vigorous feeders.

The third instar larvae spent 36 hrs in summer when the average temperature and relative humidity were 32.6 ^{0}C and 28 %.It spent 41 hrs in the rainy season when the average temperature and relative humidity were 27.5 ^{0}C and 72 %, while in winter 3rd instar spent 42 hrs when the average temperature and relative humidities were 27.1 ^{0}C and 52.5 %. The sizes of maggots reached the maximum level and started migrating away from the media for pupation.

The prepupae remained for 30 hrs in summer when the average temperature and relative humidities were 31.8 ^{0}C and 30 %, while in rainy and winter prepupae spent 73 and 48 hrs respectively when the average temperature and relative humidity in rainy and winter were 28.2 ^{0}C, 67 % and 25.6 ^{0}C and 42 % respectively. The prepupae initially started searching for a safe and dry place for pupation. The sizes of prepupae shrunk because they stopped feeding at this stage. The sizes of prepupae in rainy season were bigger than the sizes in summer and winter, which indicate that the temperature and relative humidity in the rainy season was more favorable for the larval development.

Pupae in summer season spent 102 hrs (4 days, 6 hrs) when the average temperature and relative humidity were 31.4 ^{0}C and 26 %. It spent 116 hrs (4 days ,20 hrs) in rainy season when the average temperature and relative humidity were 17.9^{0}C and 68 %, while in winter season when the average temperature and relative humidity were 24.6 ^{0}C and 40 % pupae spent 178 hrs (7 days, 10 hrs). The sizes of pupae in rainy season were bigger than the sizes in summer and winter.

After 218 hrs (9 days, 2 hrs) adult emerged out from the pupae in summer and 277 hrs (11 days, 13 hrs) in the rainy season. While in winter season, adults emerged after 317 hrs (13 days, 5 hrs). The size of adult varied in different seasons, in the rainy season the sizes were bigger than the sizes in summer and winter.

The total time spent in feeding and post-feeding stages varied in different seasons. In summer, total time spent in larval or feeding stages was 86 hrs (3 days, 14 hrs). In rainy season, total time spent in feeding was 88 hrs (3 days, 16 hrs) means delay by about one day from the time spent in summer, while in winter season were 91 hrs (3 days, 19 hrs) indicating a delay of

about one day from rainy season and two days from summer.

The developmental time of post feeding stages in summer was 132 hrs (5 days 12 hrs). In the rainy season was 189 hrs (7 days, 21 hrs) which delayed by about half day, while in winter spent 226 hrs (9 days, 10 hrs)) that indicating delayed of about two days from time spent in summer and one and a half day from time spent in the rainy season.

The total life cycle of *C. rufifacies* in summer was completed in 218 hrs (9 days, 2 hrs) when the average temperature was 31.6^{0}C and average humidity was 26 %. In rainy season it was completed in 277 hrs (11 days, 13 hrs)) when the average temperature was 29.2^{0}C and average humidity was 67 %, while in winter was completed in 317 hrs (13 days, 5 hrs) when the average temperature and humidity were 24.1^{0}C and 39 % respectively.

At 35^{0}C temperature egg, survival and development rates are maximal (Foster *et al.*, 1975; Vogt and Woodburn 1980). Usually eggs hatch within 12-24 hrs, if the oviposition site remains moist (Mackerras, 1933).The duration of the post feeding larval stage is highly variable. During summer, the median time drops off to pupariation is 2 days and ranges between 1 and 4 days (Dallwitz and Wardhaugh, 1984; Mackerras, 1933). The time to pupation increases with decreasing temperature in autumn.

Pupal development rates increase linearly between 15^{0}C (25 days) and 30^{0}C (6 days) (Foster *et al.*, 1975).Pupae held under fluctuating or constant temperature regimes show similar development rates and survival between 15 and 30^{0}C. Under fluctuating conditions pupae can survive for short exposure to -10^{0}C or 46 ^{0}C. Survival of pupae exposed daily for 7 hours to 38 and 0^{0}C, was 78 and 98%, respectively (Dallwitz 1984).The period of emergence of flies from a single cohort of post feeding larvae varies from 4 days in summer to about seven weeks in the spring (Foster *et al.*, 1978; Vogt and Woodburn, 1979).

This study supports the results obtained by Abd Al Galil, F. M. A. (2015). He has studied the seasonal effects on the life cycle of *C. rufifacies*. His study shows that life cycle of *C. rufifacies* completed in 241 hrs (10.04 days) in summer season when the average temperature ranged from 32 - 35.7 ^{0}C and an average humidity ranged from 19 - 30 %. In rainy season life cycle completed in 275 hrs (11.46 days) when the

average temperature ranged from 26.1- 29.2 ^{0}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 ^{0}C and 20 - 29 % respectively.

Conclusion:

PMI based on insect developmental rates or on insect colonization and succession patterns at carrion. An insect activity is highly influenced by temperature, which can vary, based on season and geographic location. In general, climatic conditions, particularly temperature play an important role in the insect activity, development and carrion decomposition. Variations in climatic conditions lead to differences in the decomposition speed, insect development rate and succession pattern. Results vary as per the habitats, seasons and geographic locations. Carrion decomposition studies conducted in various geographic locations and during varying season within one geographic location is, therefore, a necessity in developing baseline data for use in the field of forensic entomology.

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Table 1: Duration of different life cycle stages of *Chrysomya rufifacies* in summer and PMI in hours.

PMI in Hours	Developmental Stages	Length (mm)	Width (mm)	Weight (mg)	Temperature (°C)			Humidity (%)		
					Max	Min	Average	Max	Min	Average
0	Eggs	1.4 ±0.04	0.6 ±0.06	0.51±0.02	39.2	28.4	33.8	38	22	30
0-23	1st Instar	4.7 ±0.01	1.2 ±0.11	8.2 ±0.39	40.2	28.6	34.4	34	18	26
23-50	2nd Instar	8.9 ±0.27	2.3 ±0.39	20.3±1.54	39.6	28.6	34.1	37	17	27
50-86	3rd Instar	14 ± 0.81	3.5 ±0.11	51.2±1.10	38.4	26.8	32.6	37	19	28
86-116	Prepupae	12.1±0.28	3.8 ±0.33	42.8±0.66	38.1	25.5	31.8	39	21	30
116-218	Pupae	9 ± 0.76	3.6 ±0.47	36 ± 0.06	37.4	25.4	31.4	33	19	26
218 hrs. (9days, 2hrs).	Adult	8 ± 0.03	3.5 ±0.17	30 ± 0.85	37.6	25.6	31.6	35	17	26

± Standard Deviation for five values

Table 2: Duration of different life cycle stages of *Chrysomya rufifacies* in rainy season and PMI in hours.

PMI in Hours	Developmental stages	Length (mm)	Width (mm)	Weight (mg)	Temperature (°C)			Humidity (%)		
					Max	Min	Average	Max	Min	Average
0	Eggs	1.2 ± 0.06	0.4 ± 0.06	0.47±0.04	29.6	28.6	29.1	88	64	76
0-23	1st Instar	4.2 ± 0.64	1 ± 0.76	7.4 ± 0.29	30.1	29.1	29.6	82	66	74
23-47	2nd Instar	7.8 ± 0.22	1.8 ± 0.18	18.3±1.33	30.2	29.2	29.7	78	66	72
47-88	3rd Instar	13.4±0.35	2.7 ± 0.63	46.2±1.15	28.6	26.4	27.5	81	63	72
88-161	Prepupae	11.6±0.28	3.5 ± 0.61	40.8±1.56	29.6	26.8	28.2	77	57	67
161-277	Pupae	8.8 ± 0.51	3.4 ± 0.50	34 ± 1.12	28.2	7.6	17.9	79	57	68
277 (11days, 13hrs.	Adult	8.2 ± 0.63	3.3 ± 0.11	27 ± 0.26	30.3	28.1	29.2	78	56	67

± Standard Deviation for five values

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Table 3. Duration of different life cycle stages of *Chrysomya rufifacies* in winter and PMI in Hours.

PMI in Hours	Developmental Stages	Length (mm)	Width (mm)	Weight (mg)	Temperature (°C)			Humidity (%)		
					Max	Min	Average	Max	Min	Average
0	Eggs	1.6 ± 0.04	0.7 ± 0.08	0.54 ± 0.05	28.5	26.4	27.45	66	50	58
0-24	1st Instar	5.3 ± 0.33	1.7±0.14	9.8 ± 1.23	27.3	26.3	26.8	72	48	60
24-49	2nd Instar	9.5 ± 0.81	3±0.47	25.2 ± 1.15	29.2	24.2	26.7	65	31	48
49-91	3rd Instar	16 ± 0.30	4.2±0.55	58.5 ± 1.34	29.6	24.6	27.1	72	33	52.5
91-139	Prepupae	13.3±0.86	4±0.13	45.6 ± 0.38	28.6	22.6	25.6	53	31	42
139-317	Pupae	9.2 ± 0.54	3.8 ± 0.23	38.7 ± 1.76	27.4	21.8	24.6	46	34	40
317 (13days, 5hrs.	Adult	8.5 ± 0.93	3.6 ± 0.35	33.1 ± 0.87	27.4	20.8	24.1	41	37	39

± Standard Deviation for five values

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Fig. 1 (A-D): Graphical representation of effect of temperature and humidity on development of *C. rufifacies* in different season.

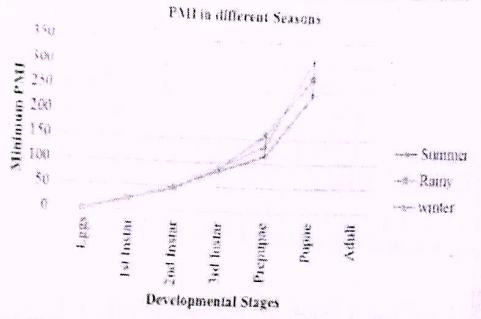

Fig 1 (A): PMI in hours in different seasons as per the development of *C. rufifacies*.

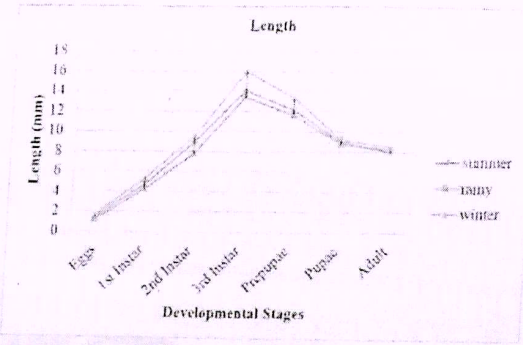

Fig 2(B): Length of developed stages of *C. rufifacies* in different seasons .

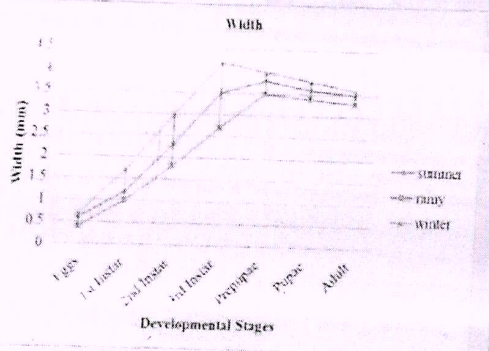

Fig 3 (C): Width of developed stages of *C. rufifacies* in different seasons.

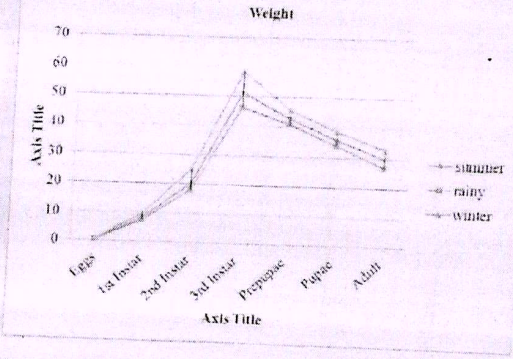

Fig 4 (D): Weight of developed stages of *C. rufifacies* in different seasons.