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On the Biology of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)

Archana Bansal
Department of Zoology, Sri Tikaram Kanya Mahavidyalya, Aligarh, U.P, India
Corresponding author: bansalarchana26@gmail.com

ABSTRACT:

Blowflies belonging to the family Calliphoridae are of considerable medical and veterinary importance. The larval forms of these flies are found in human and animal body producing a pathogenic condition known as myiasis. Keeping in view the common availability of the *C. megacephala* Fab. and its economic importance, the present study has been undertaken to acquire the comprehensive knowledge about the biology and morphology of this pest. Various species of blowflies are used as forensic indicators during crime investigation in different parts of the world. Sufficient data is still not available about C. megacephala in India, so that they can be used in forensic investigations in our country as well. For accuracy in the estimation of the Post Mortem Interval (PMI), precise developmental data of the blowfly as a forensic indicator are essential. The morphological studies were made on laboratory reared flies. Flies were reared at two constant temperatures, 25°C and 30° C for studying their biology. The incubation period of eggs at 25°C and 30°C was found to be 19.16±0.3 hrs. and 11.9±0.14 hrs, respectively. It was observed that at higher temperature, larvae took less time to emerge, which might be due to increased rate of metabolism at higher temperature. Total larval duration, pupal duration at 25°C were found to be 131.3±1.54 hrs and 126.25±1.88 hrs, respectively. These readings were decreased to 97.96± 0.47 hrs and 114.6±1.64 hrs with an increase in temperature to 30°C. Observations made on fecundity, hatchability, percentage pupation and percentage emergence at higher temperature shows an increase in comparison to lower temperature. While pre-oviposition and post-oviposition periods get decreased with increase in temperature. However, when the adult longevity is concerned, it has been observed that females live longer than males at both the temperatures.

Keywords: Blowfly, Chrysomya megacephala, Biology, constant temperature, morphology, myiasis.

INTRODUCTION

*Chrysomya megacephala F. (*the Oriental latrine fly) is a facultative myiasis producing fly. It is widely distributed throughout the Oriental and Australian regions. This species was not known in the Americas until 1975 (Imbiriba et al., 1977 and Guimaraes et al., 1978) when it was introduced into southern Brazil, probably coming from Africa (Guimaraes et al., 1980 and Laurance, 1981). Later this species made its entrance into Central and South America (Guimaraes et al., 1979; Baez et al. 1981; Prado and Guimaraes, 1982; Mariluis, 1983 and Baumgartner and Green, 1984).

Chrysomya megacephala is an important member of insect fauna of carrion. The adult female oviposits in and around the exposed wounds causing severe traumatic myiasis in humans (Sukontason et al., 2005) as well as domesticated animals. The parasitisation may result in maiming or death of the host. It can act as a vector of diseases because it is easily gets attracted to garbage and faeces. So due to this reason It also has sanitary

importance. However, despite all the calamities caused by this fly, comparatively little work has been done on its biology in India. Subramanian and Mohan (1980) and Prins (1982) have given a short account of life history of this pest. The *Chrysomya megacephala*, which belongs to the family Calliphoridae is very important as forensic indicators. The development of this fly is affected by several factors. Temperature is one of the important factors which affects its rate of development. The data which has been given by different workers shows variation in developmental periods due to variation in localized conditions. Data should be developed in different regions, in which this insect is going to be used to determine the time of death. It is essential to generate data, so that it can be properly used in forensic studies. The absence of detailed observations on this insect in Uttar Pradesh has necessitated a thorough investigation on the life history of the fly at different temperatures. It is likely, that in the near future management of the blowfly strike in flocks will depend less on insecticides and more on an understanding of the biology of the flies concerned and the factors leading to development of strikes (O'Flynn, 1982b).

Breeding technique

The adults of *Chrysomya megacephala* were collected from fields of Aligarh. Flies were maintained in cages made of fine wire mesh measuring 1' X 1' X 1' in size. They were fed on diluted milk soaked in cotton wool to which a mixture of sugar and protinex was added. Chopped buffalo meat was provided which served as an oviposition medium. Every morning the petri dishes containing diluted milk soaked in cotton swabs along with chopped meat were changed. Eggs were kept in petri dishes. Freshly emerged larvae were transferred to rearing jars 8" X 4" in size, containing chopped meat. The jars were covered over with muslin cloth so as to prevent the larvae from escaping and also to avoid external invasion. Some dry cotton was added at a later stage for the larvae to pupate therein. The pupae thus formed were sorted out and kept in meshed cloth cages.

Test methods

Observations on the biology of *C. megacephala* were made at temperatures of 25° C and 30° C. Six pairs of freshly emerged flies were obtained from laboratory reared colony. The flies were kept separately in cages of size 3" X 3"X 3" and maintained at the desired temperature in a BOD chamber. Observations were made for the incubation period, percentage hatching, larval duration, percentage pupation, pupal duration, percentage emergence, pre-oviposition, oviposition, post- oviposition periods, fecundity, longevity and sex – ratio of adults. The number of larval instars were checked by making permanent mounts of larval instars. The eggs were ruptured with the help of fine needle to study the structure of chorion under the microscope. Camera lucida was used for drawing figures. The experiment was replicated and data were analysed statistically.

Table 1: Biology of *Chrysomya megacephala* studied at different temperatures

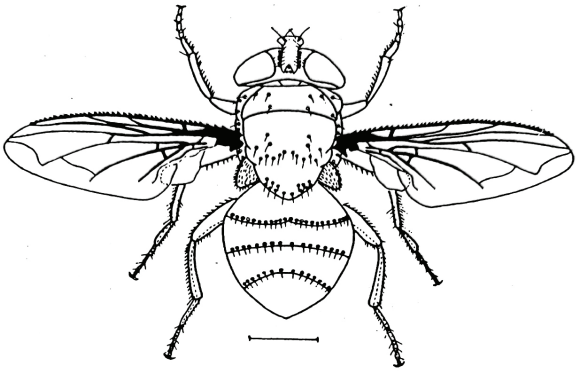
Biological parameters	Temperature 25°C	Temperature 30°C
Incubation Period (Hrs)	19.16±0.3	11.9±0.14
Duration of Larval Instars (Hrs)		
1st Instar	26.0±0.45	16.0±0.22
2nd Instar	24.16±0.16	17.2±0.3
3rd Instar	81.16±1.78	64.6±0.65
Total Larval Duration	131.3±1.54	97.96±0.47
Pupal Duration	126.25±1.88	114.6±1.64
Fecundity (no. of eggs laid /female)	642.0±11.59	758.0±12.81
Hatchability (%)	80.7±3.13	91.1±0.78
Pupation (%)	71.62±2.22	79.26±1.61
Emergence (%)	65.9±1.92	72.33±1.97
Pre-oviposition Period (Days)	7.27±0.61	4.7±0.25
Oviposition Period (Days)	40.7±1.24	28.0±1.32
Post-oviposition Period (Days)	24.7±1.35	22.2±1.24
Adult Longevity (Males)(Days)	55.98±1.16	46.9±1.76
Adult Longevity (Females) (Days)	72.7±1.15	54.9±1.60

Mean ± S.E.

Table 2: Measurements of life stages of *Chrysomya megacephala*

Stages	Length (mm)	Width (mm)
Egg	1.54±0.01	0.37±0.01
First Instar Larva	2.44±0.31	0.51±0.05
Second Instar Larva	5.36±0.68	0.85±0.11
Third Instar Larva	10.7±0.78	2.66±0.33
Pupal Stage	8.58±0.36	3.16±0.17
Adult	10.8±0.37	--

Mean ± S.E.



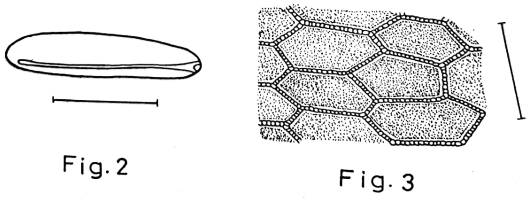


Fig. 1: Adult female (dorsal view). Scale bar = 2.7 mm.
Fig. 2- An egg. Scale bar = 900 µm.
Fig. 3: A magnified portion of the chorion of an egg. Scale bar =50 µm.

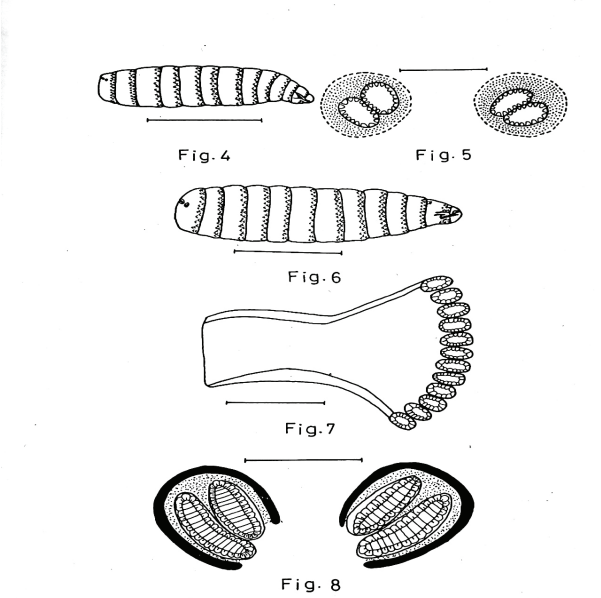
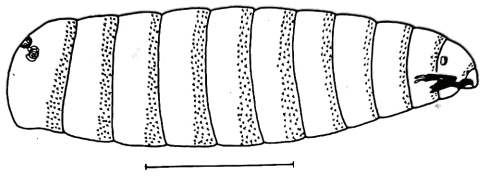
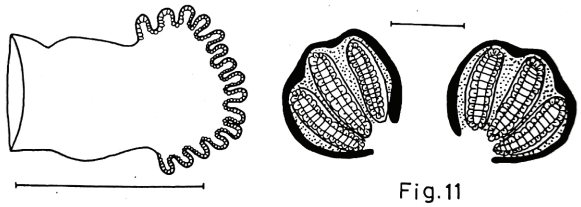


Fig. 4: First instar larva (lateral view). Scale bar = 2.2 mm.
Fig. 5: Posterior spiracles of first instar larva (dorsal view). Scale bar = 50 µm.
Fig. 6: Second instar larva (lateral view). Scale bar = 3.6 mm.
Fig. 7 - Anterior spiracle of second instar larva. Scale bar = 60 µm.
Fig. 8 – Posterior spiracles of second instar larva. Scale bar = 100 µm.





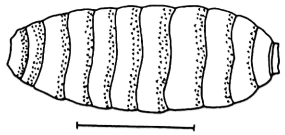
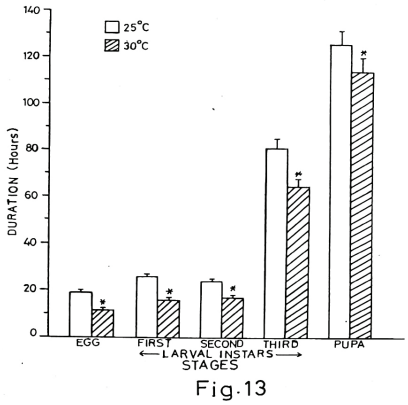


Fig. 9: Third instar larva (lateral view). Scale bar = 4.5mm.
Fig. 10: Anterior spiracle of third instar larva. Scale bar = 200 µm.
Fig. 11: Posterior spiracles of third instar larva (dorsal view). Scale bar = 200 µm.
Fig. 12: Pupa (lateral view). Scale bar = 3.6 mm.



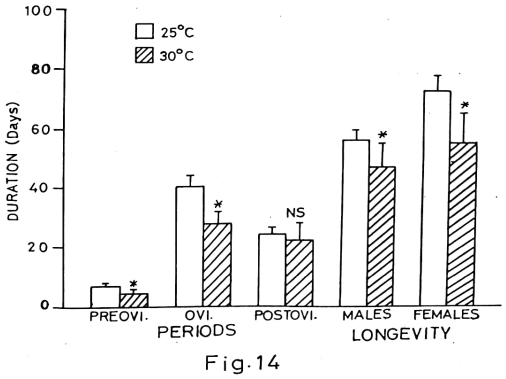


Fig. 13: Duration of different stages at 25°C and 30°C.
Fig. 14: Duration of pre-, oviposition and post- oviposition periods and longevity of male and female flies.

Table 3: Comparison of Life cycle of *Chrysomya megacephala* studied at different temperatures

Authors name	Year of study	Region	Development period	Temperature	Life span (Days)
Subramanian and Mohan	1980	India	Egg to Fly Emergence	25.6	18-19
Prins	1982	South Africa	Egg to Fly Emergence	25-28°C	11.5 – 12.75
Bharti et al.	2003	India	Egg to Fly Emergence	24.5°C	16
Bharti et al.	2007	India	Egg to Fly Emergence	25-28°	8.5-12.4
Sukontason et al.	2005	Thailand	Egg to Adult	27°C	8.5
Verma, K.	2015	India	Egg to Fly Emergence	23-27°C	8-9

RESULTS AND DISCUSSION

Mating is considered to be an essential stimulus for oviposition. Adults of blowfly *Chrysomya megacephala* start copulating after 2 to 3 days of emergence at 30°C. This period was prolonged to 3 to 4 days at 25°C. Mating began 3 days after emergence at 25°C to 30°C in *C. bezziana* (Yian & Weng, 1985) and after 2 days in *C. albiceps* (Adham & Hassan,1979). The eggs were laid intermittently at intervals of 10 seconds and were glued together in batches. A single female laid 642±11.59 and 758±12.81 eggs at 25°C and 30°C, respectively in her life time (Table 1). The number of eggs laid by *C. megacephala* at different fluctuating temperatures were 148.0±17.58 at 24-28°C and 153.33± 19.71 at 28-32°C (Claver and Yaqub, 2015). Byrd and Butler (1996) observed that females laid more eggs at higher temperature and low humidity. Female individuals mostly prefer decomposing flesh for laying eggs but it was also observed that female individuals also prefer to lay eggs on the walls of containers (Bharti et al.,2007), as has been observed in the present study. Evidences suggest that blowflies prefer diffused light for oviposition. In the present work it has been observed that flies mostly oviposit after dusk which confirms with the findings in *C. macellaria* (Dunn,1918) and *C. rufifacies* (Roy & Siddons, 1939). However, in *C. bezziana*, oviposition occurs 2-3 hours preceding dusk (Spradbery, 1979). If the egg masses were exposed to solar radiation, they suffered a significant mortality, which demonstrated a selective advantage in the timing of oviposition. The eggs were laid intermittently at intervals of 10 seconds and were glued together in batches.

The pre-oviposition, Oviposition and post-oviposition periods at 30°C were found to be 4.7±0.25 days, 28.0±1.32 days and 22.2±1.24 days, respectively (Table 1, Fig. 14). Temperature has pronounced effect on the time and duration of these periods. In the present study, the duration of respective periods showed an increase with the decrease in temperature (Table1). Claver and Yaqub (2015) made observations on pre-oviposition period in *C. megacephala* and suggested that at low temperature, egg laying time period was delayed and eggs were laid for longer time period at 24-28°C than at 19-24°C and 28-32°C. In *Lucilia cuprina*, the pre-oviposition period was five days in summer in comparison to 19 days in September (Smit, 1928). Emerged flies also showed a positive response to higher temperature as the flies laid an average of 758.0±12.81 eggs at 30°C as compared to 642.0±11.59 eggs at 25°C. Hatchability percentage was also higher at 30°C, i. e. 91.1±0.78% in comparison to only 80.7±3.13% hatchability at 25°C (Table 1). It has been reported by Davies and Ratcliff (1994) that low temperature would not support provide enough strength required for the insects to break the chorion for eclosion. The average incubation period at 25°C was recorded as 19.16 hrs with 80.7% hatching. The incubation period was reduced to 11.9 hrs with increasing hatching rate of 91.1% at 30°C (Table 1). Similar findings have been reported in *Calliphora vicina* (Reiter, 1984), *Chrysomya chloropyga putoria, Compsomyiopes boliviana, Hemilucilia flavifacies* and *Calliphora peruviana* (Greenberg & Szyska, 1984). These results support the studies made on *Protophormia terraenovae* (Clarkson et al., 2005) and *C. megacephala* (Claver and Yaqub, 2015) which stated that incubation period get progressively decreased with increasing temperature.

Pre-imaginal stages

Egg stage (Table 2, Figs. 2, 3): - The freshly laid eggs were yellowish white in colour, which turn to off white; elongate, oval in shape tapering anteriorly. Eggs measure from 1.52 to 1.58 mm in length, the average being 1.54 ± 0.01 mm. The egg is marked with longitudinal lines running parallel to each other along the whole length of the dorsal surface. The chorion is thick exhibiting conspicuous pattern of hexagonal ridges except in the region of the hatching line and the micropyle. Later it becomes transparent and the black coloured cephalic skeleton of the developing larva could be observed through the egg chorion.

The fully developed embryo prior to hatching was found exhibiting slight movement within the chorion. At this stage the egg becomes transparent and the eclosion takes place by the rupture of the anterior end. The larva wriggles out of the shell through this opening. The entire process of eclosion took 3-4 minutes.

Larval stages: - The newly hatched larvae generally feed superficially on the meat. But after few hours they burrow into the breeding medium confining themselves to the upper strata of the meat. When fully developed, the larvae leave the feeding medium move to dark and dry place for pupation. In the present study 3 larval instars have been recorded in C. megacephala. The larval duration was found to be 131.3±1.54 hrs at 25°C and 97.96±0.47 hrs at 30°C. The percentage pupation at 25°C was 71.62±2.22 as against 79.26±1.61 at 30°C (Table 1).

First instar larva (Tables 1,2: Fig. 4,5): -- The newly hatched larvae are of yellowish white colour, elongate coniform, gradually broadening from anterior to posterior end. The metapneustic larvae lack anterior pair of spiracles which distinguish them from the second instar. Each posterior pair of spiracles is formed of two elongated slits, but a peritreme is absent. At the segmental junctions the larvae are provided with a belt of backwardly projecting spines, which help in the progression of the larvae. Cephalopharyngeal skeleton is simple and unsuitable for making any appreciable dent in the intact meat and therefore, 1st instar mostly depends on liquid protein. The larvae measure 2.44±0.31 mm in length and 0.51±0.05 mm in width. The average larval duration of first instar larvae at 25°C and 30°C was found to be 26±0.45 hrs and 16.0±0.22 hrs, respectively (Fig.13).

Second instar larva (Tables 1,2: Figs. 6,7,8): -- The second instar larvae are somewhat creamy white in colour and were amphipneustic. The anterior spiracles located at the posterior end of the second segment. Each spiracle has 12 papillae arranged in a single row. Yellowish posterior spiracles placed in a deep cavity just above the anal opening. Each spiracle has two elongated spiracular slits. The inner-projection of peritreme is weakly developed and the button is wanting. Cephalopharyngeal skeleton is better developed than that of the first instar. The dentes were not fully sclerotized, but they can feed voraciously on meat. The 2nd instar larvae measure 5.36±0.68 mm in length and 0.85±0.11 mm in width. The 2nd instar larvae transformed into 3rd instar in 24.16±0.16 hrs at 25°C and 17.2±0.3 hrs at 30°C, respectively (Fig. 13).

Third instar larva (Tables 1,2: Figs. 9,10,11): -- Creamy white 3rd instar larvae have backwardly directed spines on 2 to 11 segments with 15 branches. Posterior spiracles consist of three spiracular slits. Other structures like peritreme, Cephalopharyngeal skeleton and dentes are well developed and sclerotized. Fully fed larvae after having attained their maximum size, stop feeding and start searching for a suitable place for pupation. This stage is called prepupal stage. Third instar larvae measure 10.7±0.78 mm in length and 2.66±0.33 mm in width. The 3rd instar larvae completed their development in 81.16±1.78 hrs and 64.6±0.65 hrs at 25°C and 30°C, respectively (Fig. 13).

Pupal stage (Tables 1,2: Fig 12): -- The outer covering of the prepupa hardens to form a brown, barrel shaped puparium. Two anterior pupal spiracles are short stalked and located dorsolaterally at the junction of the first and second abdominal segments. The posterior spiracles are sunken in a deep cavity. The pupae measure 8.58±0.36 mm in length and 3.16±0.17 mm in width. Pupal duration found to be 126.25±1.88 hrs at 25°C, which get decreased to only 114.6±1.64 hrs at 30°C. It shows that temperature has pronounced effect on the pupal duration as well (Fig. 13).

The third instar took longer time for development as compared to 1st and 2nd instars at both the temperatures. However, Nabity et al. (2006) and Bharti et al. (2007) observed that 2nd and 3rd instars took higher time as compared to 1st instars at constant temperature. Claver and Yaqub (2015) observed the higher stadial period for 1st instar when observations were made at fluctuating temperatures. These variations in developmental duration may be due to different methods of studies. Total larval duration showed an increase with the decrease in temperature. It shows that time period required for the formation of pupa declined with the increase in temperature as in other blowflies (Gomes et al., 2009; Claver et al. 2015). Pupal duration also showed a similar trend as found in other studies. Rate of larval and pupal development showed a direct relationship with the temperature. Which are in line with the findings of Fay (1985) and Greenberg and Szyska (1984). Higher temperature had a significant effect on percentage pupation and percentage emergence. A decrease in the mortality was observed in the present study at higher temperature.

Adult (Fig. 1): - The adult flies emerge out by breaking open the puparia. The rupture of the pupal moult is along the dorsal line of the thorax which provide a passage for adult to emerge out. The percentage emergence varied from 61.16 to 72.70 with mean of 65.9 ± 1.92 at 25°C and 64.37 to 81.58 with an average of 72.33 ± 1.97 at 30°C (Table 1).

Body of the newly emerged fly is soft and greyish in colour. In a brief period of time, the wings become fully stretched, the cuticle hardens and attains its normal colour, texture and pattern. Adults generally measure 10.0 to 12.0 mm in length, the average was found to be 10.8 ± 0.37 mm (Table 2). The size is, however, dependent upon the nutrition during its larval development of *Chrysomya megacephala* may be distinguished from other members of the family by the presence of serial hairs on the posterior side of the stem vein of the wing and the presence of fine

hairs on some portion of the upper surface of the lower calyptra.

The stoutly built flies are greenish blue with purple reflections. Both sexes bear two narrow, linear black stripes on the anterior thorax and a small black triangle situated in a posteromedial position with respect to humeral callus. The first visible segment of the abdomen is black, while the second and third segments are black banded. Sternites and edges of tergites are provided with inconspicuous golden hair. Males may be distinguished easily from females on the basis of the eyes which are holoptic and facets of upper two thirds, greatly enlarged and sharply demarcated from small facets of lower third. In females, eyes are separated by one quarter total width of head, facets uniformly small. Wings are hyaline, slightly darkened at base.

Adult longevity of males and females also get affected by the variation in temperature. At 25°C adult males and females live longer for 55.98±1.16 and 72.7±1.15 days, respectively. while at 30°C, adult males and females live for a shorter duration of 46.9±1.76 and 54.9±1.60 days, respectively (Table 1: Fig. 14). In either case females live longer than males as also observed by certain other workers. All these observations made in the present study showed that temperature has a direct effect on the blowfly *C. megacephala* development. Developmental periods were decreased with the increase in temperature thereby showing a negative correlation between longevity and temperature.

Sex ratio: - The ratio between the sexes of *C. megacephala* is determined by examining the flies, that emerged in the laboratory. The males outnumbered the females at 30°C, the ratio was 55:45 while at 25°C it was almost equal. Roy and Siddons (1939) observed a unique pattern of reproduction in C. rufifacies, that one female produces off springs of one sex only throughout its reproductive life.

The life cycle duration studied by various researchers have been shown in Table 3. Khole (1979) studied the metabolism of some calliphorids in relation to post-embryonic development and reported that metabolic rates per unit weight were found to be much higher in all metamorphic stages of the smaller species (*Lucilia cuprina* and *Chrysomya megacephala*) which had faster growth rate completing their life cycle earlier than the larger species (*Chrysomya rufifacies* and *Parasarcophaga ruficornis*) which had a delayed developmental process and lower levels of metabolism.

CONCLUSION:

This study was aimed to understand the morphology and biology of Chrysomya megacephala. From the data obtained in the present work, it is concluded that all biological parameters were affected by change in temperature. It is observed that an increase in temperature causes a direct impact on the life history of this blowfly. Environmental temperature is one of the factors for the insect development. The rate of development gets increased at the higher temperature. In the present study, the biology/ life cycle was studied at two temperatures at Aligarh, Uttar Pradesh, India. This work is of the few attempts performed earlier by other scientists in India. These findings on morphology and biology will provide a valuable information, which can be used in designing effective fly control strategies and can be a valuable forensic tool in the determination of Post Mortem Interval (PMI). The results of this study can be used in other studies in various parts of the country and will be helpful in creating general awareness about forensic entomology.

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Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

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