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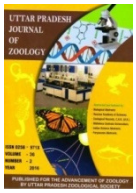

PROGRESS OF FORENSIC ENTOMOLOGY RESEARCH IN INDIA FROM PAST TO PRESENT

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AUTHORS' CONTRIBUTIONS

This research work was completed by the contribution of both the authors. Author MRA, the first author worked out according to the instructions and guidance of author SKS, the second and senior author. Author MRA is a research scholar under supervision of author SKS. Both authors read and approved the final manuscript.

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Review Article

ABSTRACT

Research on Forensic Entomology was started in 1889 in India. Several workers in India experimented on this line but the field has much more to develop. The aim of the present review is to describe different dimensions of research like Carrion Ecology Study, Forensic Entomotoxicology Study, Aquatic Forensic Entomology Study, Forensic Entomology research using morphological approach, Forensic Entomology research using molecular approach, forensically important flies on weather factors as well as seasonal variation.

Keywords: Forensic entomology research; India; review.

1. INTRODUCTION

Forensic entomology is the analysis of insect evidence for forensic and legal purposes [1]. Forensic entomology was first depicted in the thirteenth century in China by a lawyer cum death investigator Sung Tz'u in medico-legal textbook 'His yuan chi lu' (possible translation 'the washing away of wrongs'). Later on, Hoffman [2] reported insects and other arthropods as forensic indicator during mass exhumations in Germany and France. J. A. Payne [3] was supposed to be the first field entomologist of forensic entomology who performed an experiment on Pig (*Sus scrofa* L.) carrion during the summers of 1962 and 1963 in South Carolina and observed 522 arthropod species belonging to four Orders of Coleoptera, Diptera, Hymenoptera, and Araneida. Few experiments on this line were carried out by some enthusiastic persons in India. Dr. S. Coull Mackenzie, a Police Surgeon of the then Calcutta, was supposed to be the pioneering work in the field of forensic entomology in India. He described eight cases of saponification of human dead bodies in and around Calcutta in 1889. No further work on this

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aspect was reported after Mackenzie's report [4]. With the establishment of Central Forensic Science Laboratory at Kolkata in 1957, research on Forensic Entomology was developed slowly. Several workers from that time have performed different kind of researches under the broad field of Forensic Entomology up to recent times that may be categorised into six different dimensions like:

a) Carrion Ecology Study,
b) Forensic Entomotoxicology Study,
c) Aquatic Forensic Entomology Study,
d) Forensic Entomology research using morphological approach,
e) Forensic Entomology research using molecular approach,
f) Forensically important flies on weather factors as well as seasonal variation.

All the segments pointed out in the present review work, fall under the vast area of research of forensic sciences related to entomology. Undoubtedly every segment of study aims to investigate postmortem interval very minutely following its methodology which is described in brief to illuminate the affair.

a) Carrion Ecology Study: It is the entomofauna study on a decomposing animal or human dead body. In the field of Carrion Ecology, larval stages, pupal stages of different developmental maturity and adults from human dead bodies or animal carcasses are usually collected. Various stages of decomposition viz. fresh, bloat, active decay, advanced decay and dry remains of the corpse are observed. Some workers mentioned a detailed list of insects involved in each phase of decomposition. Developmental stages of maggot were observed by Rao et al. [5] to measure the time since death. With increasing interest of the study of insect behaviour on cadavers, several researchers like Kulshrestha and Chandra [6] studied various environmental conditions and faunal role on a dead body. Antemortem wounds which became unidentifiable were also observed by them. The authors described 25 cases of cadavers related to maturation of blowflies. In another investigation, Kulshrestha and Satpathy [7] evaluated Post Mortem Interval (PMI) by collecting beetles *Dermestes maculatus* (Coleoptera, Dermestidae) and *Necrobia rufipes* (Coleoptera, Cleridae) from two dry female skeletal remains. In a sensational death case of Ex-minister of Karnataka, probable PMI was determined by Kulshrestha and Satpathy [8] examining samples of fly eggs, first instar and second instar maggots on a dead body. The entomological method was superior and statistically more reliable than other medico-legal approaches in the estimation of PMI that was proved by Kashyap and Pillay [9] by examining sixteen insect-infested cadavers. Various cases of insect succession were studied by many workers. Different species of blowflies (Diptera, Calliphoridae) viz. *Calliphora vicina, C. vomitoria, Lucilia illustris, Chrysomya megacephala, C. rufifacies* were collected by Singh and Bharti [10] from rabbit carcasses in the field and various putrefying cadavers in many places of Punjab in different seasons. In another study, eight species of ants viz. *Camponotus compressus*, *Crematogaster contemta*, *C. hodgsoni* , *Dorylus labiatus*, *Meranoplus bicolor*, *Messor barbarus*, *Paratrechina longicornis*, *Tapinoma melanocephalum* (Hymenoptera, Formicidae) were observed by Singh and Bharti [11] from rabbit carrion in the field during various seasons of the year.

Three species of Muscidae and one species of Sarcophagidae were recorded by Bharti et al. [12] from India for the first time from carrion of rabbit in Patiala campus of the Punjabi University of state Punjab. Muscids viz. *Hydrotaea occulta*, *H. capensis*, *Atherigona savia* and *Sarcophaga princeps* were recorded. To show nocturnal oviposition behaviour of blowfly, an experiment was conducted by Singh and Bharti [13] around fourteen night trials at an unilluminated area in Patiala by taking mutton as a bait on the top of a pole and 3 types of Calliphorids (*Calliphora vicin*a, *C. megacephala, C. rufifacies*) laid eggs in five cases. It was rather an extension of an experiment designed by Greenberg [14]. A similar type of study was performed by Singh and Bharti [15] at an unlighted area in Patiala, Punjab taking a piece of mutton as bait on the top of a pole to observe nocturnal larviposition behaviour of flesh flies in September 2005. It was found that *Sarcophaga albiceps* and *S. hirtipes* were able to deposit their larvae at night also. In an elaborated successional study, a total of 38 insect species belonging to four orders and thirteen families (Calliphoridae, Sarcophagidae, Muscidae, Anthomyiidae, Otitidae, Staphylinidae, Histeridae, Cleridae, Dermestidae, Tenebrionidae, Silphidae, Formicidae, Tineidae) were collected by Bharti and Singh [16] on decaying rabbit carcasses and it was surveyed that maximum diversity of insect species occurred in Spring. Twenty cases of death were studied by Aggarwal et al. [17] to monitor the insects involved in the decay of corpses. Gupta and Setia [18] suggested that Forensic entomology was utilised to determine PMI, the season of death, geographical location of death, movement or storage of remains after death etc. and molecular identification was also exposed a new arena in that field. Singh and Sharma [19] criticised forensic research in India. They remarked that lack of baseline data and experienced professionals for measurement of minimum post-mortem interval made the line most untouched till date. The baseline data were mainly

involved in taxonomy, zoogeographic distribution, biology and ecology of insects. Singh and Bala [20] surveyed the time length of the starvation period required to induce post feeding behaviour shortened with increasing age of larvae.

A brief overview of the necessity of forensic entomological data in a criminal investigation was provided by Joseph et al. [21]. These data were related to insects and their larval morphology, growth histories, species distribution and toxic contents in their tissue. An initiative was taken by Goyal [22] to measure the postmortem interval observing members of Calliphoridae, Sarcophagidae, Muscidae, Silphidae, Dermestidae at different decomposition stages on 47 human corpses in Amritsar, Punjab. The PMI of a headless 9 months old male foetus was calculated by Babu et al. [23] finding in the forest nursery at Jagdalpur on the basis of the developmental period of second instar larvae of blowfly *C. rufifacies* utilising Accumulated Degree Hours(ADH) method. The death time duration of human corpses was estimated by Chandna [24] measuring length of maggots of house fly (*Musca domestica*), flesh fly (*Sarcophaga* sp), skipper fly (*Piophila casei*) (Diptera, Piophilidae) and blowfly (*Calliphora vomitoria*) with scale in millimetres in Karnal, Haryana. Bala and Kaur [25] observed insects from buried remains. For that purpose, an experiment was conducted on a buried piece of pork at a depth of 30 cm around 16 days in Ghawaddi village of Ludhiana in the state of Punjab and ten species of beetles belonging to 6 families and 2 species of order Hymenoptera were observed by them. Insect succession on the same piece of pork was observed by Bala and Kaur [26] that was buried in the soil after 48 hrs of insect exposure in the same place. In that case, 41 insect specimens belonging to 13 species under 9 families of the order Diptera, Coleoptera and Hymenoptera were monitored. Singh et al. [27] criticised that many research papers and review articles remained about terrestrial forensic entomology, but few numbers were available on buried dead bodies. So, they reviewed the works to estimate post-burial interval, its importance and future perspective. A case study was done by Bala and Sharma [28] on a decapitated 38 years aged male human dead body at Punjab. Few third instar larvae of *C. megacephala* were collected from the amputated arm of the corpse, and they calculated the number of days required for *C. megacephala* to develop from egg to larvae. Tentative PMI was estimated at 10.6°C and according to them, that was 9 days. Bharti [29] had taken an initiative to find out the correlation between faunal composition and change in altitude in Himalayan regions. For that purpose, a study was conducted at a different elevation in North West Himalaya using carcasses of a cow as bait.

It was reported that blowfly diversity was decreased rapidly at an altitude of 2511m showing only three species due to limited abiotic factors and *C. villeneuvi* was abundant at that elevation. *C. megacephala* and *C. rufifacies* were monitored at first three elevations (350m, 970m and 2057m) because having extreme tolerance capacity of temperature fluctuations and flies belonging to genus *Calliphora* were present at higher altitudes of 2057m and 2511m because those were cold resistant. In a similar type of study, 19 species of blowflies belonging *to Chrysomya, Lucilia* and *Calliphora* were observed by Jadav and Sathe [30] in the Western Ghats. They also reported that the number of *Lucilia* and *Chrysomya* were more in low altitude. The authors further analyzed that diversity of blowflies was decreased with increasing altitude. A study was performed by Singh et al. [31] on thirty-eight human corpses to estimate the PMI using statistical tool SPSS version 20. It was observed that time duration for egg laying was 1-3 days. In another study, 25 species of forensically important 12 families of order Diptera were reported by Sathe et al. [32] from Western Maharashtra, India using pig liver as bait or from dead bodies. Later, the succession and life cycle of *Dermestes maculatus* was studied on exposed piglets' carcasses by Sonker et al. [33]. Fly specimen was abundant during spring and summer. In a different study, *S.* (*Liosarcophaga*) *dux* was reported by Chakraborty et al. [34] for the first time in India on a corpse of house gecko *Hemidactylus flaviviridis*. Another experiment was performed by Chakraborty et al. [35] on the cadavers of *Gallus gallus* around three different seasons including pre-monsoon, monsoon and post-monsoon to observe the effect of seasonal variation on development of immature stages of *S.* (*Parasarcophaga*) *albiceps* using one way ANOVA. It was found that there were no significant differences in the average length, average width and average biomass of the immature stages in seasonal variation. In the same year, another study was performed by Das et al. [36] on the carcasses of white rat (*Rattus rattus*) in West Bengal. They reported *Musca domestica, Lucilia cuprina* and *Hemipyrellia ligurriens* in it. Later, a study was conducted by Singh et al. [37] on laboratory-bred rat (Rattus *norvegicus*). Nine species of flies belonging to four families, viz. Calliphoridae (44.24%), Sarcophagidae (16.53%), Muscidae (28.87%) and Phoridae (13.35%) were observed and *P. ruficorni*s firstly arrived and *M. domestica*, *C. megacephala* remain throughout the experiment, whereas *Megaselia sp* was present on the third day.

The PMI from larvae of *C. megacephala* was determined by Sharma et al. [38] using base temperature or lower threshold temperature in ADH method. In another work, Sharma et al. [39] suggested

various methods for determination of PMI of Calliphorid flies and also analysed problems facing by forensic entomologists while measuring the time since death. Sharma and Singh [40] critically evaluated the progress of forensic entomology from past to present days emphasising PMI and insect succession in different countries adding Indian scenario also. Past and present overviews of India in the field of forensic entomology were given by Sharma and Singh [41]. The PMI of a mummified body of 23 year old female by observing the developmental time of *C. megacephala* from egg to pupa utilizing Accumulated Degree Hours (ADH) at 25±1°C, was estimated by Bala and Sharma [42]. Their estimated PMI value was 9.6 days. A case study was done by Sharma and Bala [43] on 45 years aged male dead body in Ludhiana, Punjab, India. PMI was determined to utilise ADH method. Dynamics on human corpses by local insects like *C. megacephala, C. rufifacies, Dermestes maculatus, Necrobia rufipes, Phormia regina, C. albiceps* were observed by Sharma et al. [44]. The insects were collected from five dead bodies involving different case studies in Punjab. In another case, a study was performed by Bala and Singh [45] using the meat of goat (*Capra* sp) in the summer of 2014 and 2015 from Himachal Pradesh and Punjab to know the geographical distribution of some forensically important seven species of beetles belonging to family Silphidae in North India. *C. saffranea* was observed by Abd-AlGalil et al. [46] for the first time from Aurangabad, India. Third instar larvae of *C. saffranea* were collected by them from decaying body of cats from the garden of Zoology Department at the Dr Ambedkar Marathwada University reserve in Aurangabad, Maharashtra and then the larvae were reared under laboratory conditions to become adult. Lastly, flies were identified by morphological features. They also suggested that blow flies were forensic bioindicators and those flies played a vital role to estimate PMI. In another study, an unknown skeleton was studied by Kumar and Gupta [47]. Then, the life history of *S. ruficornis* was studied by Adhikari et al. [48] providing chick liver as bait. It was observed that the protein content of liver was decreasing throughout the decomposition and the protein content of developing stages of the fly increasing linearly. An investigation was done by Juglan et al. [49] on 100 human dead bodies at the Mortuary of Gandhi Medical College, Bhopal, India to monitor the effect of temperature and humidity on the estimation of PMI by using entomological procedures and finding its relationship with autopsy-derived postmortem interval. They noticed 78.2% Calliphorid, 12.6% Sarcophagid, 3.1% Muscid flies, 1.7% Dermestid and 1.2% clerid beetles on human corpses. It was reported that 66 % cases showed the same range of data between the entomologically derived PMI and PMI obtained from routine autopsy. In another work, Juglan et al. [50] reported the activity of insect occurrence on the human dead body. Insects associated on carcasses were Calliphorids (78%), Sarcophagids (13%), Muscids (3%) and a small number of Beetles, cockroaches and red ants also. *C. megacephala* was found in 55.2% cases and *C. rufifacies* in 43.2% cases in Calliphorid population. It was monitored the maximum activity of insect occurrence in the rainy season (July to September). An experiment was designed by Hore et al. [51] on Indian mole-rat (*Bandicota bengalensis*) carcasses to monitor dipteran species composition and its succession patterns simultaneously in two different localities- one in the urban locality and another in a suburban locality under the metropolitan area of Kolkata, during the month of April 2017. They noticed eight species belonging to three families in urban and six species belonging to four families including one new in suburban areas. It was surveyed that Muscids were dominant colonisers in urban habitat whereas in the suburban area overall uniformity in terms of species richness and abundance in all the families were found. In the context of colonization on corpses in the urban locality, *Synthesiomyia nudiseta* (Muscidae) arrived firstly and *Atherigona orientalis* (Muscidae) lastly whereas in suburban locality *C. rufifacies* (Calliphoridae) was appeared as first species and *Megaselia scalaris* (Phoridae) as last species.

b) Forensic Entomotoxicology Study: This is generally carried out when necessary tissues of corpses were not obtained to conduct toxicological study [52] or at that situation when blood, urine or internal organs were absent for criminal investigation [53]. A study on forensic entomotoxicology was conducted by Gola and Lukose [54] to determine the presence of chemicals or drugs in insect body. For that purpose Librium (chlordiazepoxide), Diazepam, Prednisolone, Phenobarbitone and DDT were used as ante-mortem administered drugs or chemicals. They found the presence of Librium (chlordiazepoxide), Diazepam, Prednisolone and Phenobarbitone in all the three larval stages, pupal stages and adult form of blowflies but DDT was absent beyond the larval stages.

Verma [55,56] investigated life cycle pattern of *C. rufifacies* under four different temperatures like Cool temperature (Humid 20-24°C), Cool temperature (Dry 18-22°C), Room temperature (Humid 26-30°C) and Room temperature (Dry 24-28°C) and effect of two drugs ethanol and cannabis on the development of it. It was observed that higher temperature and humidity accelerated the growth and maturation. According to them, ethanol and cannabis showed

faster development than the control condition. Singh et al. [57] conducted an experiment by rearing *C. megacephala* on rat tissues administering half lethal (37.5 mg/kg bw), lethal(75 mg/kg bw) and twice lethal (150 mg/kg bw) dosages of Ketamine hydrochloride previously to monitor the effect on the development of fly taking different parameters like weight, length, width, time of adult emergence and survival rate. In another study, Singh and Bhupinderjit [58] reared larvae of *C. megacephala* on rat tissues exposed to Cadmium chloride (CdCl2) in three different concentrations viz. half lethal (3.25mg/kg bw), lethal (6.5 mg/ kg bw) and twice lethal (13 mg/kg bw) by intraperitoneal injection to observe the effect of Cadmium chloride on development of *C. megacephala.* It was monitored that larval development was required longer time. In high cadmium concentrations, the body weight of larvae, pupa and adult flies were reduced in association with greater mortality rate. In a study, larvae were collected by Sallawad et al. [59] from the carcass of dog and reared on beef liver containing Paracetamol, Caffeine and Codeine. They investigated the effect of the drug on the lifecycle of blowflies under the concentration of 1 gram of drug / 1 kg meat. According to them that Paracetamol showed the neutral effect on blow flies, Caffeine extending the lifespan of the flies and larvae did not survive in Codeine.

c) Aquatic Forensic Entomology Study: This is related to the estimation of PMI in case of carcasses found in the submerged condition in aquatic sources. Very few works on aquatic forensic entomology have done in India till recent time. Many papers were available on forensic entomology in terrestrial habitat but very little works were done in an aquatic habitat. A review paper about aquatic forensic entomology pointed out only its importance, future prospective and forensically important insects in the aquatic environment were published by Sharma and Singh [60]. Another experiment on this line was designed by Singh and Greenberg [61] by submerging pupae of known ages of five species of blowflies one to five days duration underwater to know chances of survival. It was observed that 25% of pupae of *Protophormia terraenovae, Phormia regina, and Phaenicia sericata* (Diptera, Calliphoridae) generated normal adult flies after 4 days of immersion but none after 5 days. They also reported the expansion of pupal period among survivors occurred in relation with duration of submersion. According to Singh et al. [62] maggots of *C. megacephala,* Sarcophagids and diatoms conjointly helped in PMI estimation. They also help to know the drowning cause of death simultaneously by taking the data from two case studies. Maggots were cultured in laboratory and diatoms were collected from bone marrow of human corpses and also from the site of drowning. Later, in context of establishing a relationship between submergence period and survival rate of fly maggots, Singh and Bala [63] observed that during immersion of larvae of 10 hrs (more than 2 hrs of submersion); 20 and 30 hrs (more than 3 and 4 hrs of submersion); 40, 50, 60 and 70 hrs (5 hrs of submersion) survival rate was 33% in first case and no one survived in the last two cases. According to them, such type of findings may be useful for determination of minimum time since submergence (TSS) of a dead body during a forensic investigation.

d) Forensic Entomology research using morphological approach: Accurate morphological identification of immature or adult stages of insects indicates one of the paramount factors for forensic investigation because it indirectly helps us to determine postmortem interval. Identification of species of Calliphoridae was done by Greenberg and Singh [64] through analysing the morphology of eggs of its 11 species belonging to six genera with SEM study. Ultrastructural study of first, second, third instars and puparium of *P. ruficornis* were observed by Singh et al. [65] using light microscopy and scanning electron microscopy for the first time in India. Main diagnostic characters, i.e. cephalopharyngeal apparatus, cephalic segment, structure and orientation of spines, pupal respiratory horns, structures of both anterior and posterior spiracles were considered by them. Those characters are certainly helpful in forensic investigations of fly species. Bala and Sharma [66] commented that traditional techniques of PMI of blowflies in most cases were accurate but lack of precision. So, they mentioned some recent techniques of blowfly age estimation that was pteridine fluorescence analysis, cuticular banding pattern analysis, internal morphological analysis, cuticular hydrocarbon analysis, gene expression analysis, volatine organic compounds analysis released by larvae and pupae. Sharma and Singh [67] suggested various morphological and molecular bases of identification methods for forensically important Indian flies. According to them, morphological methods were a study of Allozymes, Cuticular hydrocarbon techniques and Scanning Electron Microscopy whereas techniques using Nuclear DNA, Nuclear Ribosomal DNA and Mitochondrial DNA as markers were included in molecular-based identification methods. In another case, SEM studies on the morphology of sensilla of antenna of adult male and female of *H. ligurriens* were observed by Hore et al. [68] for the first time. That finding helped easier identification of insect morphology from the forensic

entomological viewpoint and better distinction of *H. ligurriens* from other calliphorids.

e) Forensic Entomology research using molecular approach: Identification of insect specimens through morphological approach is confusing in some instances. Having similar characteristic features of some individuals, a parallel molecular-based identification method was evolved to simplify the identification process using various molecular markers like Cytochrome Oxidase subunit I (COI) and subunit II (COII), NAD Dehydrogenase subunit 5 (ND5), 16S rRNA. In India, molecular Study in the field of forensic entomology was done on Sarcophagid, Calliphorid and very little on Muscid flies using mitochondrial genes [69] and 16S rRNA as a marker. According to Bajpai and Tewari [70] two mitochondrial genes Cytochrome Oxidase subunit I (COI) and NAD Dehydrogenase subunit 5 (ND5) were helped to know genetic relatedness and to construct phylogeny among five sarcophagid species using PCR. In another study, five sympatric species of the genus *Sarcophga* was described to determine genetic relationship using mitochondrial COI and II region [71]. Identification of forensically important fly *C. megacephala* and *C. rufifacies* was done to observe polytene chromosomes from trichogen cells remain in the pupae [72]. Molecular identification of three Sarcophagid flies was carried out by Sharma et al. [73] based on 450 bp region of Cytochrome Oxidase I gene. DNA amplification study was done by Khullar and Singh [74] from 13 to 14 years old insect samples of four species viz. *C. vomitoria*, *H. pulchra*, *Catapicephela splendens* and *Catapicephela ingens* (Diptera, Calliphoridae) using 16S rRNA primer. It was observed that CO1 gene was highly fragmented but the 16S rRNA gene remained well protected from fragmentation and gave ideal results for old samples.

Sharma and Singh [75] suggested that morphological identification of the immature stages of Calliphoridae was difficult when only fragmented specimens are available. Molecular phylogenetic analysis of *C. rufifacies* and *C. megacephala* was performed by them using 480 bp mitochondrial cytochrome oxidase I (mtCOI) gene sequence as a marker. By taking 450bp fragment of COI gene of mitochondrial DNA from ten Sarcophagids, identification and phylogenetic analysis were done by Sharma et al. [76]. Later, molecular study and construction of the phylogenetic tree of six blowflies were done using 350 bp COI gene of mitochondrial DNA by Khullar et al. [77]. Sharma and Singh [78] suggested that DNA based methods were useful in case of unavailability of the sample or in case of torn and ruptured sample for identification purpose. They reviewed different DNA based methods like PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), Random Amplified Polymorphic DNA (RAPD), PCR-Amplified Fragment Length Polymorphism (PCR-AFLP) and DNA Sequencing for identification of forensically important flies up to species level. Insect succession studies and molecular identification studies of beetles were critically examined by Bala and Singh [79]. According to Sharma and Singh [80] RAPD was needed in those cases where earlier nucleotide sequence data was absent. They reviewed many cases where RAPD were used to characterise forensically important flies. Then, phylogenetic analysis was conducted by Khullar et al. [81] using Cytochrome oxidase II (COII) gene of four forensically important blowflies. The phylogenetic relationships were determined by them on the basis of four different approaches: UPGMA, Neighbour-joining, Maximum Likelihood and Minimum Evolution. In the same year, Barcoding of Cytochrome C Oxidase Subunit I (COI) of mitochondrial DNA was conducted by Algalil and Zambare [82] to carry out molecular identification of three Sarcophagids. In a study, DNA based identification of nine species of blowflies belonging to three subfamilies Calliphorinae, Luciliinae, and Chrysomyinae was performed by Bharti and Singh [83] using COI gene as a molecular marker. Molecular identification and construction of the phylogenetic tree of five muscid flies of Indian origin were done by Singh and Achint [84] using 500-520 bp mitochondrial COII gene as a marker. Average haplotype diversity and nucleotide diversity that were 0.833 and 0.02547 respectively within the different species was calculated by utilising Dna SP Version 5.0.

f) Forensically important flies on weather factors as well as a seasonal variation: Temperature and Humidity have great roles in the development of insects. Season also shows pronounced impact on life cycle duration of insects. A study was done by Karunamoorthy and Lalitha [85] in which they reared larvae of forensically important Australian sheep blowfly *Lucilia cuprina* on lean beef and adults on a glucose-nutrient broth mixture, lean beef and sugar. Life spans of both adult male and female were from 23 to 31 days at 32 ± 2°C. Another study was conducted by Bharti and Singh [86] on the carcasses of domestic rabbits to observe various larval stages of three blowfly species viz. *C. vicina*, *C. megacephala* and *C. rufifacies*. According to the authors, *C. megacephala* and *C. rufifacies* were found to breed around the year, whereas *C. vicina* was able to breed on carcasses only during winter and spring season. Then, the development time of *C. megacephala* at four different temperatures 22°C, 25°C, 28°C, 30°C at laboratory environment was investigated by

Bharti et al. [87] and it was reported that there was an inverse relationship between temperature and time of development. The time periods required to complete lifecycle of the fly were 15.5 days, 12.4 days, 8.5 days and 6.3 days at 22°C, 25°C, 28°C and 30°C temperatures respectively. Bharti [88] found a correlation between temperature and time required to complete life cycle of *C. vicina* and *M. domestica nebulo* and it was concluded that when the temperature was increased, days required to complete life cycle became shortened. The life cycle of *P. ruficornis* in the rainy season was studied by Patil [89]. It was observed that maggots were required four days to reach pupal stage and pupa was converted to adult in eleven days. So, the whole life cycle of the fly was completed in sixteen days according to the author. It was also suggested that the life cycle study of *P. ruficornis* for PMI estimation was done scarcely in India than Europe, America and Australia. In another study, *C. megacephala* and *C. rufifacies* were surveyed at four different temperatures (22°C, 25°C, 29°C and 31°C) in order to reach an interrelationship between larval age, body length and body dry weight [90].

Chakraborty et al. [91] studied the attack of parasitoid *Brachymeria minuta* (Hymenoptera: Chalicididae) on pupal development of the flesh fly *S. albiceps* in pre monsoon, monsoon and post-monsoon. According to them, percentages of host specificity and average yearly parasitoidism were 65.62% and 50.93% respectively. The time duration required to complete the life cycle of *P. albiceps* was extended from 324 ± 12 hrs to 479.6 ± 48.2 hrs due to parasitoid attack and parasitoidism was highest in summer. They suggested that since the immature stages of the fly were required to estimate the PMI, so this data might be utilised in the future as a reference. Later, Chakraborty et al. [92] suggested that immature stages of blowflies were needed to determine PMI. A study was designed by them in which *Gallus gallus* Corpses were taken to observe the development of two Indian blow flies *C. megacephala* and *L. cuprina* for pre-monsoon, monsoon and post-monsoon using one way ANOVA as a statistical tool. According to them, *C. megacephala* might be used for estimation of PMI in pre-monsoon , monsoon seasons because of better growth in warm and moist weather whereas post monsoon was the best season for *L. cuprina*. In another case, the developmental rate of *L. sericata* and *C. megacephala* was investigated by Verma and Paul [93] on beef liver taking as bait at laboratory environment. They reared *L. sericata* at temperatures between 22°C and 26°C (mean 24°C) and relative humidity 50% ±10% and *C. megacephala* at temperatures between 23°C and 27°C (mean 25°C) and relative humidity 55% ±10%. It was monitored that life cycle of *L. sericata* and *C. megacephala* were completed in 10-11 days and 8-9 days respectively. According to them, that data would be helpful to forensic investigators as a future reference. Bansode et al. [94] suggested that in case of estimation of PMI of insect species, the developmental stage of insects and surrounding temperature were considered as major elements. They also added that duration of life cycle stages was dependent on temperature. An experiment was designed by them in which *P. ruficornis* larvae were reared in the incubator. It was found that *P. ruficornis* developed normally up to 35°C but mortality occurred at higher temperatures. However, at 40°C the rate of development was very high but at the low-temperature time required to complete life cycle was very long. In another case, nearly similar type of study was done by Bansode et al. [95] on another forensically important fly *L. cuprina*. A similar type of findings was observed in that case. In that case time required to complete the life cycle of the fly was 627 h (26 days) in 20°C, 531 h (22 days) in 25°C, 333 h (14 days) in 30°C, 287 h (11 days) in 35°C and 267 (11 days) in 40°C. Again, life cycle duration of the *L. cuprina* was investigated by Bansode et al. [96] in different seasons as well as its morphological parameter also. According to Abd-AlGalil et al. [97] temperature and humidity were major factors taking great roles in larval development, decay and degradation of the corpses. It was monitored that the life cycle of *C. saffranea* was completed in 220 hours in summer, in 259 hours in the rainy season and 341 hours in winter. In the same year, Lifecycle duration of two forensically important flies *C. megacephala* and *C. rufifacies* were investigated by Siddiki and Zambare [98] in laboratory condition in different seasons. It was found that *C. megacephala* was taken 237.47 hrs, 263.51 hrs and 211.13 hrs to complete its life cycle during rainy, winter and summer seasons respectively whereas *C. rufifacies* was required 239.14 hrs, 286.02 hrs and 216.26 hrs during rainy, winter and summer season respectively for completion of the life cycle. The larval growth of *S. dux* was observed by Babu et al. [99] under outdoor ambient temperature in two seasons spring and summer. From findings, it was inferred that high temperature and low humidity accelerated larval and pupal development. The required time durations to complete life cycle of *C. vicina* and *L. cuprina* were studied by Bhosale and Bhosale [100] under fluctuating temperature in summer.

2. DISCUSSION

Different dimensions of forensic entomological research are highlighted and from this discussion, it is evident that major forensic entomologists in India

engaged themselves in Carrion Ecology study. Some workers focused more on the determination of post submergence interval and post-burial interval also. Pankaj Kulshrestha, the forensic specialist of Medico-Legal Institute of Bhopal, is a pioneer in this area and various workers of Punjabi University also have done a tremendous job. Many workers also engaged themselves to observe the development of insects in consequence of temperature and humidity factors in association with seasonal variations. But other dimensions of Forensic Entomology like Forensic Entomotoxicology Study, Aquatic Forensic Entomology Study, Post-burial interval Study and forensic entomology research using morphological approach are not properly excavated. Forensic entomology research using molecular approach has been initiated in the recent decade and major portion of works has been done on Sarcophagids, Calliphorids and very little on Muscids. More emphasis should be given to forensic entomology research in India in future.

3. CONCLUSION

Forensic entomology study has many dimensions which were studied in India by different workers at different times. Research on this field has not been done up to the mark till date. Forensic entomology research using morphological approach has to be developed more in India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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