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"Analysis of Substrate Preference of Blow Fly Pupation: Implications for Postmortem Interval Estimation"

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Abstract:
Problem- The aim of this study was to assess the impact of substrate texture and composition on the development time and survival rate of blow fly pupae. **Approach-** Blowfly pupation behaviour has been studied in extensive detail, this preliminary study examined Chrysomya megacephala (Fabricius) preference for substrate, vertical dispersal behaviour, and fly emergence rate under a variety of natural environmental parameters and hard and soft soil conditions. Third instar larvae were collected from two distinct soil samples to investigate the substrate preference of Chrysomya megacephala and their dispersal behavior in response to natural environmental conditions. **Result-** The maximum depth at which Chrysomya megacephala pupae are found is 2 cm, with a maximum size of 0.8 cm. Typically, the orientation of the pupa within the soil is vertical. In hard soil characterized by low humidity and moisture content, blow flies exhibit a prolonged emergence period from the pupa stage. Conversely, in soft soil with high humidity and moisture levels, blow flies tend to emerge earlier. **Conclusion-** Observations indicate that temperature plays a significant role in the life cycle of these flies. Lower temperatures extend the duration of the life cycle, while higher temperatures shorten it. Variations in temperature and humidity directly impact growth and indirectly affect the estimation of postmortem interval. Therefore, to enhance the accuracy of such estimations, the historical data of temperature and humidity in the vicinity where the body was discovered should be taken into account.

Keywords- Chrysomya megacephala**,** Substrate preference, Pupation behavior, Postmortem interval

Introduction:

Forensic entomology is a study of insects arrived on the decaying carrion and has a significant impact on the estimation of minimum postmortem interval (Babu et al. 2022). Calliphorids, commonly known as blow flies, play crucial role in the decomposition process of animals. Blow flies are the first to arrive at a dead body in less than 10 min and lay their eggs within an hour (Sharma et al. 2021) on decomposing remains to aid in criminal investigations. The eyes, mouth, or ears are the main areas where blow flies deposit their eggs, as these areas are well protected for the developing larva. The larvae pass through three instars until they stop feeding during the second half of the third instar and mostly leave the cadaver for pupation (Baqué et al. 2015) and they can be found in the soil between 3-5 cm below the surface (Byrd and Brundage, 2020). The pupal stage is an important step for the life cycle of holometabolous insects (Kökdener and Sahin Yurtgan, 2022) because the pupal stage of blow flies is about 50% of the immature development. However, age estimation of pupae is more difficult compared to the age estimation of larvae due to the lack of observable morphological changes without a complicated preparation of the puparium or a time-consuming further rearing up to the adult fly (Hartmann et al. 2023). When third instar larvae fully grown, they stop feeding and depart from the cadaver to pupate. The larva contracts during pupariation, and its outer layer solidifies into a puparium that resembles a barrel. The insect goes through a metamorphosis inside this puparium, becoming a pharate adult after first changing into a pupa (Martín-Vega et al. 2017).

The blowflies substrates in which they develop are distinct and ephemeral, so in larval stage blowflies face restricted food resources. These substrates are usually colonized by one or more species of insects, there is often enormous competition for resources. Once the food resources become depleted, the larvae leave in search of a place to pupate, or for another source of food if they have not achieved the minimal weight required for pupation. This process is known as post-feeding larval dispersal. During this process, the larvae have to deal with various abiotic changes, with temperature and photoperiod being considered the most important (Gomes et al. 2006) (Gomes, Gomes, and Von Zuben 2009). Additional factors that have been observed to influence developmental time of blowflies include tissue type, and behavioral factors (e.g., competition and predation) (Bauer et al. 2020).

Ascending and descending vertical dispersal Ascending

Horizontal dispersal refers to a common phenomenon in which third instar larvae, at a specific stage in their development, leave their food substrate and move along the surface. This movement is typically part of their life cycle and is essential for finding new food sources or suitable environments for further development. It allows them to explore their surroundings and avoid overcrowding or competition for resources in their current location. Vertical dispersal occurs when larvae bury themselves in the soil to undergo pupation. After completing their development, adults emerge from the soil ready to continue their life cycle, such as mating and laying eggs to start the process anew. This vertical movement from underground to the surface allows the insects to transition from their immature stage to their adult form in a suitable environment for their next life stage (Sharma et al. 2021).

Material and Method:

The puparia utilized in this study were sourced from Kurali Chandigarh Rd, Mullanpur, Punjab. A portion of the puparia was meticulously collected from the site using forceps or a brush. These puparia typically exhibit a grayish-brown coloration [Fig: 1].

The pupae are found around the remain stage of carcass. Appropriate ambient temperature and humidity levels were recorded prior to pupae collection. Following this, blow fly pupae, approximately 1 inch in depth, were discovered in the soil in a vertical orientation [Fig: 2]. The soil exhibits a moist and soft consistency due to the presence of carcass dung. The pupa is retrieved using forceps and transferred into a plastic tube, where its size is measured in the laboratory using measuring scales [Fig: 3]. The pupae were observed daily and adults were collected and identified with the help of taxonomic keys by (Ramaraj et al. 2014).

Fig: 1. Grayish brown pupa

Fig: 2. Vertical orientation of Pupa

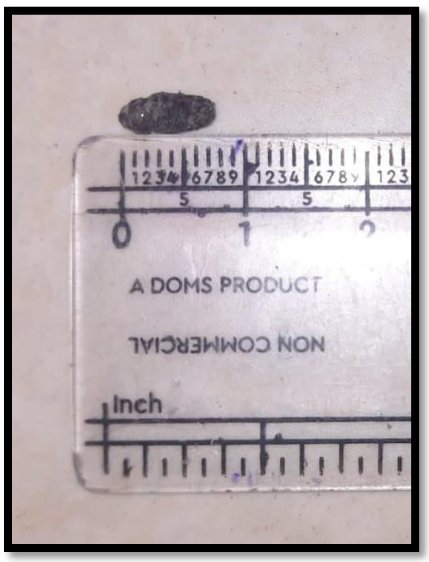

Fig: 3. Adult pupa size

Results- The pupation study was conducted under natural weather conditions at the actual site of occurrence. The maximum temperature recorded was 35°C with a minimum of 25°C; humidity ranged from a maximum of 58.6% to a minimum of 47.2%, and soil pH ranged from a maximum of 8 to a minimum of 6.

Morphological Features- Head: Eye facets of upper two-thirds greatly enlarged and sharply demarked from small facets of lower third. Antenna short, postpedicel four times length of pedicel, pedicel dark brown, third brownish; arista dark brown, long plumose; palpi yellowish and slender. Thorax: Prostigmatic bristles present; anterior and posterior thoracic spiracles dark brown. Wings: Stem-vein with a row of setulae on upper posterior side; upper surface of lower lobe of squama hairy; upper squama white, partly dark margined; lower squama brown. Abdomen: Genital tergites bluish with black hairs; inner forceps elongated, outer forceps reduced; anterior paramere wide, slightly curved anteriorly; posterior paramere bifurcated at end; acrophallus wide at end and with a wide stalked projection posteriorly [Fig:4].

Fig: 4 Adult blowfly

Table 1: Pupa to adult stages in soft soil sample

Pupa to adult stages of Ch. Megacephala	Length (cm)	Time Spent in Stage(Days)	Temperature °C	Humidity	Soil Moisture %	pH of soil
Initial stage of pupa	0.29	17/03/24	30°C	36%	50	7
	0.34	18/03/24	31°C	37%	55	8
	0.57	19/03/24	31°C	39%	60	6.6
Adult stage of pupa	0.81	20/03/24	32°C	36%	70	7

In loose or soft soil, pupation in blow flies tends to occsur more rapidly compared to compacted soil due to the favorable conditions provided by elevated temperature, humidity, and soil moisture levels.

Table 2: Pupa to adult stage in hard soil sample

Pupa to adult stages of Ch. Megacephala	**Length (cm)**	**Time Spent in Stage(Days)**	**Temperature °C**	**Humidity**	**Soil Moisture %**	**pH of soil**
Initial stage of pupa	0.24	11/04/24	37°C	29%	45	7
	0.43	13/04/24	34°C	34%	40	6.5
	0.63	15/04/24	34°C	52%	40	6
Adult stage of pupa	0.78	17/04/24	32°C	29%	37	7

After 5 to 6 days of pupation, the adult blow fly pupa reaches the appropriate size and undergoes emergence. In compacted soil, pupation may experience a slight delay due to reduced humidity and soil moisture levels.

These environmental factors significantly influence the developmental rate of the pupae, accelerating the transformation process. Loose soil, with its higher permeability and aeration, allows for better heat retention and moisture distribution, creating an optimal environment for pupation. Elevated temperatures play a crucial role in increasing metabolic rates within the pupae, thereby speeding up their development. Additionally, the high moisture content in soft soil helps maintain the necessary hydration levels for the pupae, preventing desiccation and ensuring continuous development. Under warmer temperatures, the enzymatic and biochemical processes within the pupae are enhanced, leading to quicker maturation. The presence of sufficient moisture further aids in this process by providing a stable environment that supports the pupae's physiological needs. As a result, blow fly pupae in these conditions can complete their maturation cycle more swiftly than those in compacted soil.

Discussion-

Puparia can be found on the clothing of the deceased or in close proximity to a cadaver during an investigation. As the longest stage, the pupal stage is important for estimating the postmortem interval (PMI). Soil-dwelling insects' population dynamics, survival rates, spatial distribution, and developmental processes are significantly impacted by environmental factors as soil type and moisture content. Particularly in clay soil, the maximum pupal developing length was recorded at 100% soil moisture, while the shortest period was recorded at 25% soil moisture. Different moisture content levels did not consistently affect pupation development in the current investigation. Variations in the characteristics of the soil, such as the amount of organic matter, its ability to retain water, and the sizes of its particles, could be the cause of this observation (Kökdener and Sahin Yurtgan, 2022). The kinds and diversity of insect species present, as well as their seasonal availability, are definitely greatly influenced by the soil types (Byrd and Brundage, 2020) and local weather circumstances.

In this investigation, we assessed the vertical dispersal behavior of feeding third instar larvae and the eclosion success of Chrysomya megacephala. Over 80% of the larvae exhibited vertical dispersal, with pupation occurring at depths not exceeding 2cm (Sharma et al. 2021).

Sukontason et al. (2008) examined how changes in temperature within a range, with an average of 27°C, affected the development of C. megacephala from newly hatched larvae to pupariation in the months of June, July, and September. The hours for June, July, and September were 96, 108, and 168 hours, respectively. Furthermore, (Babu et al. 2022) carcasses decompose more quickly in the summer than they do in the winter due to higher temperatures. Temperature affects insect development; warmer temperatures quicken the succession process by increasing the development of immature insect stages. In warmer weather, fly larval activity is increased; in colder weather, it is inhibited. Rising temperatures cause an increase in metabolic rate, which accelerates development. Chrysomya megacephala was found to develop during a period of seven consistent temperatures (16°C, 19°C, 22°C, 25°C, 28°C, 31°C, and 34°C). This study offers important information for using C. megacephala in forensic death investigations. Our results offer useful approximations and insights into the ways in which different soil types and moisture contents can impact the life cycle of C. megacephala. They also provide pertinent information about the differences in pupal survival under natural environmental conditions.

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