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A Study on Pupation of *Calliphorid* and *Sarcophagid* Towards Different Applied Substrates

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Abstract: Different substrates were used to evaluate the pupation site preferences of *Calliphorid* and *Sarcophagid* flies released in two batches of "10" and "50" during the months of October and December. Data were recorded for fifteen minutes for consective five days. In the month of October batches of "10" were found most attracted by the feathers (15.8±6.165 day^{-1}) and least by the bread crumb (4.6±6.885 day^{-1}) while the batches of "50" showed more response towards soil (15.4±6.897 day^{-1}) and least towards the bread crumb (4.8±6.709 day^{-1}). During the month of December, most and least attractive substrates for the batches of "10" and "50" were recorded the cotton (14.0±6.898 day^{-1}) and feathers (15.6±5.485 day^{-1}), respectively. Pupation substrates showed statistical significant variations ($p<0.001$) between them. Months did not differ significantly ($p>0.001$). By comparing both batches and months it was concluded that feathers (15.8±6.165 day^{-1}) proved the most attractive substrates while bread crumb (4.4±2.108 day^{-1}) least preferred site for pupation.

Key words: Pupation · Attraction · Substrates · Blow · Flesh · Flies

INTRODUCTION

Blowflies, flesh flies, beetles, mites, moths and bacteria are dominant groups of organisms which play a very important role in decomposition of materials of plant and animal origin. A number of non-feeding flies are scavengers in nature but the most important among these are blow flies (Calliphoridae*)* and flesh flies (Sarcophagidae). These flies, especially, play a much important role in decomposition. Pupae of both flies are resistant to environmental extremes. Immature stages of flies as post feeding larva or pre-pupa disperse to find suitable site to pass pupal stage.

The members of Calliphoridae are called the blowflies. Their life cycle, generally, completes within 9-21 days and come in four stages. Larvae or maggots are also white to yellowish when fully grown, they move around the corpse as a group and pre-pupae migrate away from corpse seeking a suitable pupation site. Blowfly's pupae are encased in light brown to black puparia, whereas, adult flies are recognized by their metallic colors ranging form black, blue, copper and Green [1]. Blowflies often found from large to moderate in size. Adult blowflies feed primarily on flower nectar, plant sap and other surgery materials [2]. The members of Sarcophagidae are also called flesh flies. These are similar to blow flies in both larval and adult habits. Flesh flies generally complete their life cycle within 8-21 days. Larvae of flesh flies resemble the larvae of blowflies and go through three larval instars. The larval skin during the pre-pupal stage contracts and hardens into a protective shell. This shell is called a puparium. Adult flies are grayish with three black strips running the length of the top surface of thorax [1].

The adults of these flies are known to be of great medical and veterinary importance due to their role in myiasis in wild and domestic animals [3]. Blow flies and flesh flies as hosts for different parasitoids are discussed. Maggots are also used in food production. Larvae are introduced to cheese at the time of advanced level of fermentation and for breaking down the cheese's fats. Maggots can cause allergic reactions, toxicity, stomach, intestinal problems, diarrhea, vomiting, nausea and

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dysentery. Immature stages of blow and flesh flies are one of the primary invertebrate consumers of decomposing animal organic matter and human dead body [4]. Larvae or maggots migrate away from corpse in order to find suitable site to pass pupal stage. This pupal stage is extremely important in forensic entomology. So it is necessary to investigate pattern of larval dispersion on the pupation site. The pupation site preference has been studied because of number of larvae pupated at different sites in the culture [5].

MATERIALS AND METHODS

The experiment trials were conducted in open field of Zoology Department at G.C. University, Faisalabad. The pre-pupae of *Calliphorid* and *Sarcophagid* flies were secured from the chicken corpse put in a blowfly trap [6]. Different pupation substrates were placed in the circular manners with few inches distance. The responses of pre-pupae of C*alliphorid* and S*arcophagid* flies were observed towards different pupation substrates. The pre-pupae were released in two batches of "10" and "50" pre-pupae in the center of a four feet diameter hard board. The experiment was conducted in two months (October and December) in order to observe the difference with the reference to environmental factors changing in both months and to confirm the data recorded. The choice, considered to be made when pre-pupae reached a particular pupation substrate, was recorded. It was also observed, for fifteen minutes, that whether the choice made by pre-pupae was temporary or permanent. The number of pre-pupae making particular choices was recorded. There were six pupation substrates viz. Soil (SOL); Semi-burnt substrate (SBS); Feathers (FTR); Cotton (CTN); Bread crumb (BRC) and cracked bark (CBR).

The observations were made daily from 9:00 am to 11:30 am up to five consecutive days. The whole experiment was repeated in October and December in order to determine their responses on dispersal behavior of pre-pupae. A plastic bucket of 15 inches depth was also used during the experiment in order to avoid the loss of pre-pupae or post feeding larvae by putting the trap inside the bucket. Data was analyzed by using three factor factorial (batches, pupation substrates and months) and completely randomized design [7]. Analysis of variance (ANOVA) was employed for the data recorded and differences among means were determined [8].

RESULTS

Abundance of pre-pupae of *Calliphorid* and *Sarcophagid* flies preferring a particular pupation was observed against six different pupation substrates. These substrates were commonly found in the vicinity of human habitats. This experiment was designed to trace the forensically important pre-pupae with reasonable accuracy. Variations among the all six pupation substrates were differing significant ($p<0.001$). Significant variations were observed between the batches ($p<0.01$) but significant differences ($p>0.001$) were not found between the months (Table 1). Interactions between pupation substrates and batches (PSXB) and batches and months (BXM) showed significant variations ($p<0.001$) between them and interaction between pupation substrates and months (PSXM) exhibited the non-significant variations ($p>0.001$) between them. Second order interactions among pupation substrates, batches and months (PSXBXM) revealed the statistical significant differences ($p<0.001$) among them (Table 1).

Cotton and soil substrates attracted (26 d^{-1}, 25 d^{-1}) about the same number of pre-pupae in the 1st and 2nd day. Semi-burnt substrate (54 d^{-1}) also attracted the same number of pre-pupae from 2nd to 5th day. Inconsistent patterns of attractions for five days were observed in the cases of cotton bread crumb and cracked bark. Minimum (zero) and maximum (26 d^{-1}) pre-pupae recorded for the soil and cotton during the 1st day, respectively. Similarly persistent increasing patterns were observed for cotton and breadcrumb while reverse was true for soil. Maximum (7 5d^{-1}) and minimum (23 5d^{-1}) pre-pupae were observed for the cases of feathers and breadcrumb and differed significantly ($p<0.001$). Statistically significant variations ($p>0.001$) did not found for soil, cotton and bread crumb (Table 1). Feathers (79 5d^{-1}) proved most attractive pupation site and least (23 5d^{-1}) for bread crumb (Table 3).

Responses of both flies towards various substrates were observed. Progressive increasing or decreasing patterns of attractions did not found for all pupation materials. Only increase in pre-pupae was seen in the case of breadcrumb from the 2nd day to 4th day. Significant fluctuations were recorded for all pupation substrates during the whole period of study (Table 3). Maximum and minimum prepupae were seen in the case of cotton and bread crumb, respectively. Significant variations ($p<0.001$) found between them (Table 1). Pre-pupae hiding under the

Table 1: Total number of pre-pupae of five days; mean values per day (mean±SD) and batches of "10" and "50" during the month of October.

	BATCHES OF "10"							BATCHES OF "50"						
P.S.	D1	D2	D3	D4	D5	T	Mean±SD	D1	D2	D3	D4	D5	T	Mean±SD
SOL	0	25	15	10	11	61	12.2±7.477ab	8	21	22	16	10	77	15.4±6.897a
SBM	14	10	10	10	10	54	10.8±1.552bc	7	6	16	6	9	44	8.8±6.638cd
FTR	5	11	10	16	14	79	15.8±6.165a	20	12	0	18	12	62	12.4±6.714bc
CTN	26	1	0	17	15	59	11.8±7.156bc	25	13	9	12	14	73	14.6±6.739b
BRC	5	1	3	4	7	23	4.6±6.885d	9	2	6	7	0	24	4.8±6.709e
CBR	12	18	13	15	10	68	13.6±6.710a	14	10	6	13	13	56	11.2±6.508c
CV(%)		LSD		vb_1			b_2	CV		LSD		vb_1		b_2
12.40		10.201		-0.6331			0.1888	10.201		8.210		-0.6331		-0.7010

Soil (SOL); semi-burnt material (SBM); Feathers (FTR); cotton (CTN); bread crumb (BRC); cracked bark (CBR); D, days; mean values followed by same alphabets do not differ at (p≥0.01); T, total pre-pupae of five days; $\sqrt{b_1}$, Skewness (lack of symmetry in data distribution); b_2, Kurtosis (showing peak and length of tail in data distribution); P.S. pupation substrate

Table 2: Total number of prepupae of five days; mean values per day (mean±SD) and batches of "10" and "50" during the month of December.

	BATCHES OF "10"							BATCHES OF "50"						
P.S.	D1	D2	D3	D4	D5	T	Mean±SD	D1	D2	D3	D4	D5	T	Mean±SD
SOL	12	14	13	14	12	65	13±6.3230b	16	12	14	13	15	70	14±05.631a
SBM	11	8	12	10	9	50	10±6.1220c	12	13	11	12	13	61	12.2±5.509b
FTR	14	12	13	16	15	70	14±6.0080a	17	14	16	14	17	78	15.6±5.485a
CTN	16	16	14	11	13	70	14±6.8980a	16	14	15	16	11	72	14.4±5.525a
BRC	4	3	8	7	9	31	6.2±5.8540d	5	6	6	0	5	22	4.4±02.108d
CBR	12	12	8	9	10	51	10.2±2.753c	2	12	10	8	12	51	10.2±5.338c
CV (%)		LSD		vb_1		b_2		CV		LSD	`	vb_1		b_2
8.717		9.741		-0.5223		-0.7498		10.404		10.140		0.344		1.387

Soil (SOL); semi-burnt material (SBM); Feathers (FTR); cotton (CTN); bread crumb (BRC); cracked bark (CBR); mean values followed by same letters do not differ at (p ≥0.01) ; D, days; T, total prepupae of five days; $\sqrt{}$, Skewness (lack of symmetry in data distribution); Kurtosis, b_2 (displaying peak and tail of data distribution); P.S. pupation substrate

Table 3: Analysis of variance (ANOVA) of data for pupation site preference of *Calliphorid* and *Sarcophagid* pre-pupae towards different substrates in the batches of "10" and "50" during the months of October and December.

SOV	df	SS	MS
Batches (B)	1	3.1250	3.125***
Pupation materials (PM)	5	1353.45	270.692***
BXPM	5	50.792	10.158***
Months (M)	1	2.3470	2.347^{NS}
BXM	1	1.1250	1.125^{NS}
PMXM	5	48.903	9.781***
BXPMXM	5	59.792	11.958***
Error	48	107.333	2.236
Total	71		

***, Significant differences (p<0.001); NS, non-significant differences (p>0.001)

soil (15.4±6.897 day^{-1}) and cotton (14.6±6.739 day^{-1}) did not differ significantly (p>0.001). Similar cases were observed for feathers and cracked bark. Cracked bark attracted same no of pre-pupae for 4th and 5th day and maximum attraction found as 56 5day^{-1}. In the batches of "50" during October, soil (77 5d^{-1}) proved cist preferable then bread crumb (24) (Table 3).

Considerable increasing trends were observed for breadcrumb and cracked bark while decreasing for soil and cotton substrates. Fluctuations patterns were seen in all the remaining materials. About same pre-pupae were attracted by the feathers and cotton pupation materials. Maximum (70/5d) attractions were found in the cases of feathers and cotton respectively while minimum (31/5d) for bread-crumb material. Remaining and cotton substrates attracted same number of pre-pupae and found non-significant variations (p>0.001) between them (Table 1). Highest attraction (70 5d^{-1}) recorded for the feathers and cotton and least (31 5d^{-1}) attracted by the bread crumbs material (Table 4). During December in the batches of "50" different pupation substrates exhibited different patterns if distribution. Soil and semi-burnt material contained considerable increasing trends while consistent patterns were observed for the bread crumb material. Maximum number of pre-pupae found for the feathers and cotton in the 3rd and 4th day of study (Table 2). As a whole maximum (78 5d^{-1} and minimum (22 5d^{-1}) number of pre-pupae maximum recorded for the feathers and breadcrumb in the month of December and

significant variations were observed between them. In the contrary, values did not show any significant differences ($p > 0.001$) for soil, feathers and cotton. During the month if December in the batches of "50" feathers (78 $5d^{-1}$) found best for pre-pupae and least bread crumb (22 $5d^{-1}$).

DISCUSSION

Adult of blow and flesh flies are known to be of the great medical and veterinary importance due to their role in the myasis in wild and domestic animals [3]. Myasis in humans commonly detected in neck or scalp regions, but may occur in other parts of body as well. Serious chronic effects and death have resulted from infestation of the nose, eyes, ears and mouth. Immature stages of both flies might be attacked by parasitoids or predators like ants, spiders and crows. Several species of blowflies and flesh flies transport a variety of gastrointestinal pathogens and are carrier of number of diseases. In the medical research maggots therapy has been used for various purposes like by dissolving infected tissues, wound disinfection by killing the bacteria and stimulating the wound healing. For wound repair and regeneration maggots theraphy have been used for various purposes as reported by Sherman [10]. This therapy has also been investigated in United State for wound healing and repairs in horses since 2003.

General behavior and status in various animals of blow and flesh flies had been studied by Shah and Sakhawat [6]; Wells and Kurahashi [11]; Aguiar and Milward [12]; and Tachibana and Numata, [13]. Various environmental factors including temperature and photoperiods are seriously involved in the dispersion of the post feeding larvae. Dispersion and pupation site preference was studied by Ghafoor *et al*. [14]. Life history, behaviour and pupation site preferences of both flies were observed by various researchers [5, 6, 14]. Several species of blowflies and flesh flies transport a variety of gastrointestinal pathogens and are carrier of number of diseases. Isolation and detection pathogenic H5N-1 Avian Influenza a virus from blowflies were collected from vicinity of infected poultry farm in Kyoto, Japan in 2004. Same species were used in the current experiment to evaluate the responses for pupation sites preference by applying various pupation substrates. Insects play much important role in determining the postmortem, interval and location of death [3]. Analysis of environmental factors particularly photoperiod and temperature can be helpful in searching for dispersing of larvae around cadavers [15]. Larval and pupal behaviors of blow and flesh flies were observed by many investigators [1, 5, 12]. Similarly, the research on pupation site preference of blow and flesh flies were also carried out by numerous eminent researchers [5, 14, 16, 17]. In the present research on various pupation substrates larvae in batches of "10" and "50" were released and their dispersal behaviors was observed during the months of October and December. Goff, [18] pointed out that blowflies arrived within minutes to several hours after death depending on environmental conditions but flesh flies arrived at the same time or several hours after blow flies. During the month of October feathers (79 $5d^{-1}$) proved the most preferred substrate for pupation but Yasin [5] found that soil and semi-burnt material was the most preferred pupation site. Various researchers proved that that bark was the most attractive site for pupation [2,16,17]. Sometimes *Calliphorid* and *Sarcophagid* flies like the soil for residing due to its soft nature. Dry leaves and newspapers provided the moderate attraction for pre-pupae [16, 17]. Similar findings from various experiments were also obtained by Yasin, [5]. Larvae mostly tend to pupariate near the food sources. Goday *et al*. [7] Pointed out that in most of the cases maggots preferred food materials for pupation but Ghafoor *et al*. [17] proved that decayed fruits were best residing site for pupation. In the batches of "50" (October) soil (77 $5d^{-1}$) attracted maximum number of pre-pupae on the contrary Yesin, [8] declared that soil was the moderately attractive site for pupation.

In the present research during the month of December, batches "10", feathers and cotton (70 $5d^{-1}$) attracted same and maximum number of pre-pupae and bread crumb proved least attractive site for pupation. Ghafoor *et al*. [17] found fresh leaves were the best materials which attracted maximum number of maggots. In the batches of "50" feathers proved best preference material for the residing of maggots but most of the authors [6, 16, 18] studied that bark was the most preferred material for the pupation. In the resent study, pre-pupae moderately preferred the cracked bark for their pupation. Ghafoor *et al*. [17] investigated that folded clothes were the least attractive pupation material but dry leaves and newspapers were the moderate attractive sites for pupation. It is a need of time to explore the general behavioral, dispersal patterns and various aspects of post feeding larvae of blow and flesh flies. In future eminent researchers should be encouraged to investigate about the medical importance of C*alliphorid* and *Sarcophagide* flies.

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