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MORNING RESPONSES OF *CALLIPHORID* AND *SARCOPHAGID* PREPUPAE TOWARDS DIFFERENT PUPATION MATERIALS

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ABSTRACT

An experiment was conducted to evaluate the pupation site preference of *Calliphorid* and *Sarcophagid* flies towards the various pupation materials in the months of October and December. Flies were released in the two batches of "10" and "50". Six pupations materials were the news paper strips, fresh leaves, dry leaves, decayed fruits, decayed vegetables and folded clothes. Data were recorded for fifteen minutes for five consecutive days. Results showed that decayed vegetables proved the best sites for pupation in both batches "10' and "50" respectively during the month of October. In the month of December decayed fruits and fresh leaves proved the most preferred site in the batches of "50". and "10" respectively. In both months and batches folded clothes found the least attractive site for pupation.

Key words: - morning response, *Calliphorid, Sarcophagid,* pupation materials

INTRODUCTION

Blow flies, flesh flies, beetles, mites, moths and bacteria are dominant groups of organisms involved in deposition. A number of non-feeding flies are scavengers in nature but the most important among these are blow flies (*Calliphoridae)* and flesh flies (*Sarcophagidae*).

Calliphoridae: The members of *Calliphoridae* are also called Blow flies. Life cycle of Blow flies generally completes within 9-21 days and come in four stages (Goff, 2000 and Saferstein, 2004). Larvae or maggots are also white to yellowish when fully grown, they move around the corpse as a group and prepupae 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 (Bryd and Castner, 2001). These flies are large to moderate in size. Adult blowflies feed primarily on flower nectar, plant sap and other sugary materials (Subramanian and Mohan, 1980).

Sarcophagidae: 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 prepupal 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 (Bryd and Castner, 2001).

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 (Zumpt, 1965). 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 dysentery. Immature stages of blow and flesh flies are one of the primary invertebrate consumers of decomposing animal organic matter and human dead body (Greenburg and Kunich, 2002)

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 on the basis of number of larvae pupated at different sites in the culture (Yasin, 2004).

MATERIALS AND METHODS

The responses of prepupae of C*alliphorid* and S*arcophagid* flies were observed towards different pupation materials during present study. The experiment was conducted in open field of Zoology Department in G.C. University, Faisalabad. The prepupae of *Calliphorid* and *Sarcophagid* flies were secured from the chicken corpse put in a blowfly trap (Shah and Sakhawat, 2003). The prepupae were released in batches of "10" and "50" in the center of a four feet diameter hard board. The choice, considered to be made when prepupae reached a particular pupation material, was recorded. It was also observed, for fifteen minutes, that whether the choice made by prepupae was temporary or permanent. The number of prepupae making a particular choice was recorded. There were six pupation materials newspapers

(NP); fresh leaves (FL); decayed leaves (DL); decayed fruits (DF); decayed vegetables (DV) and folded clothes (FC) released in two batches "10" and "50" during the months of October and December.

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 prepupae. A plastic bucket of 15" depth was also used during the experiment in order to avoid the loss of prepupae or post feeding larvae by putting the trap inside the bucket.

Statistical analysis: Data were analyzed by using three factor factorial and completely randomized design (CRD) (Steel *et al.*, 1997). Differences among means were ranked using LSD Test.

RESULTS AND DISCUSSION

Dispersion of larvae for pupation site preference: Abundance of prepupae 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 prepupae with reasonable accuracy.

Preference in the batches of "10" in October. The results showed (Table 2,Fig; 1,a) that most preferred pupation material was decayed vegetable followed by fresh leaves, decayed leaves, decayed fruits and news papers while folded clothes showed the least attraction for pupation. Folded cloths, decayed leaves and decayed fruits exhibited moderate attraction for pupation. The total number of prepupae (80, 89) attracted by the fresh leaves and decayed vegetables respectively were almost the same ($p \geq 0.01$). The prepupae (65, 26) attracted by dry leaves and folded clothes showed wide differences between them. Similar trend of differences was also observed between the decayed vegetables and folded clothes. The prepupae (80, 89) attracted by fresh leaves and folded clothes exhibited least differences ($p \geq 0.01$) between them. Similar trend was showed in the decayed fruits and decayed leaves (Table 2, Fig1, a).

Preference in the batches of "50" in October. It was found from the results that most attractive materials were the decayed vegetables and fresh leaves. The dry leaves exhibited the moderate attraction for pupation while least preferred site was the folded clothes. The number of prepuae (85, 59) attracted by the decayed vegetables and news paper strips were differing significantly ($p \leq 0.01$) and similar trend was observed in the values of news paper strips and folded clothes (59, 20) (Table-2, Fig1, a).

Preference in the batches of "10" in December. In the month of December it was observed that fresh leaves, decayed leaves and decayed fruits found the same pupation site while decayed vegetables showed slight difference. The prepupae (35) attracted by folded clothes proved the least attractive site for pupation. The numbers of prepuae (70, 70) attracted by dry leaves and folded clothes were exactly the same and did not differ significantly ($p \geq 0.01$). Statistically when we analyzed the data significant differences ($p \leq 0.01$) were observed between the number of prepupae (73, 35) attracted by the fresh leaves, folded clothes. The folded clothes proved the least preferred site for maggots (35). The news paper strips and decayed vegetables showed the moderate attractions for maggots (50, 65) ($p \geq 0.01$) (Table 3, Fig 1, b).

Preference in the batches of "50" in December. It is evident from the data that decayed vegetables were the most favorable material for the pupation followed by the decayed fruits and fresh leaves. Moderate attraction was showed by the dry leaves and news paper strips while lowest of prepupae (16) were attracted by the folded clothes. Significant differences ($p \leq 0.01$) were observed between the prepupae (91, 16) attracted by the decayed vegetables and folded clothes similar situation was observed for decayed vegetables and folded clothes ($p \leq 0.01$) (Table 3, Fig 1, b).

Fresh leaves were the most favorable site for pupation in December because kairomones are emitted by leaves proved the attractive cue for pupation. Statistically it was found that data of both months was not differing significantly ($p \geq 0.01$) (table 1). In the experiment two types batches "10" and "50" of fruit and flesh flies were used to analyse pupation site preference. Significant differences ($p \leq 0.01$) were observed between two batches (table 1). Six types of food sources and vegetative materials were used for the study of pupation site preference. Different types of substrates showed different types attractive behavior. So when we analyzed this data significant difference (($p \leq 0.01$) among pupation materials were found (Table 1, 2, 3 Fig 1 a, b).

When we compared both months and batches it was observed that maggots (91) preferred the decayed vegetables for pupation while folded clothes attracted the least prepupae (16) in the batch of "50" during the month of December (Table 1,2,Fig 1).

Several species of blowflies and flesh flies transport a variety of gastrointestinal pathogens and are carrier of number of diseases. Detection and isolation of highly pathogenic H5N-1 Avian Influenza a virus from blowflies was 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 by applying various pupation materials. Maggots therapy for wound repair and regeneration was

reported by Sherman (2002) and this therapy have been used in United State for horses since 2003.

Table 1: Analysis of variance (ANOVA) of data for morning responses towards different pupation materials, prepupae batches of "10" and "50" during the months of November and December

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Factor P.M.	5	2037.067	407.413	164.3348**
Factor B	1	12.033	12.033	4.8538**
P.M.XB	5	83.767	16.753	6.7576NS
Factor M	1	0.833	0.833	0.3361**
P.MX M	5	53.567	10.713	4.3213***
BXM	1	4.800	4.800	1.9361ns
P.M.BXMC	5	57.800	11.560	4.6629***
Error	96	238.000	2.479	
Total	119	2487.867		

Coefficient of Variation: 9.56% LSD: 3.025
Factor-1, batches (B) ; factor-2 , pupation materials (P.M.); factor 3 , months (M); AXC ,BXC, AXBXC ,interactions;
,*, Significant at 0.01and 0.001 levels respectively; N,NS, significant ,non significant respectively.

Insects play much important role in determining the postmortem, interval and location of death. (Bryd and Castener, 2001). Analysis of environmental factors particularly photoperiod and temperature can be helpful in searching for dispersing larvae around cadavers (Gomes and Von Zuben, 2004 Feng *et al.*, 2002 and Grassberger , 2004). Larval and pupal behavior of blow and flesh flies was observed by many investigaters (Goff, 2000; Feng *et al* 2002; Gomes *et al.*, 2005; Gomes and Von Zoben, 2005). The work of pupation site preference by prepupae of blow and flesh flies was carried out by Yasin, 2004; Akbar, 2005 and Arshad, 2005. In the current experiment on various pupation materials larvae in the batches of 10 and 50 and their dispersal behaviors was observed during the months of October and December. Goff (2000) pointed out that blow flies 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. Leaves were the most preferred materials for pupation because moisture plays a very prominent role in keeping the prepupae out of heat, but Yasin (2004) found that soil was the most preferred pupation site. In some investigation it was proved that back was the most attractive site for pupation (Akbar 2005 and Arshad 2005). Sometimes *Calliphorid* and *Sarcophagid* flies like the soil for residing due to its soft nature. Dry leaves and newspapers provided the moderate attraction for prepupae. Similar results were obtained by Yasin (2004). Sometimes decayed fruits were moderately preferred by the prepupae for pupation due to their medium level of affinity for the subtract.

Table:-2 Total number of prepupae in 5 days; (mean±SD) mean value /day and batches of "10" and "50" during the month of October

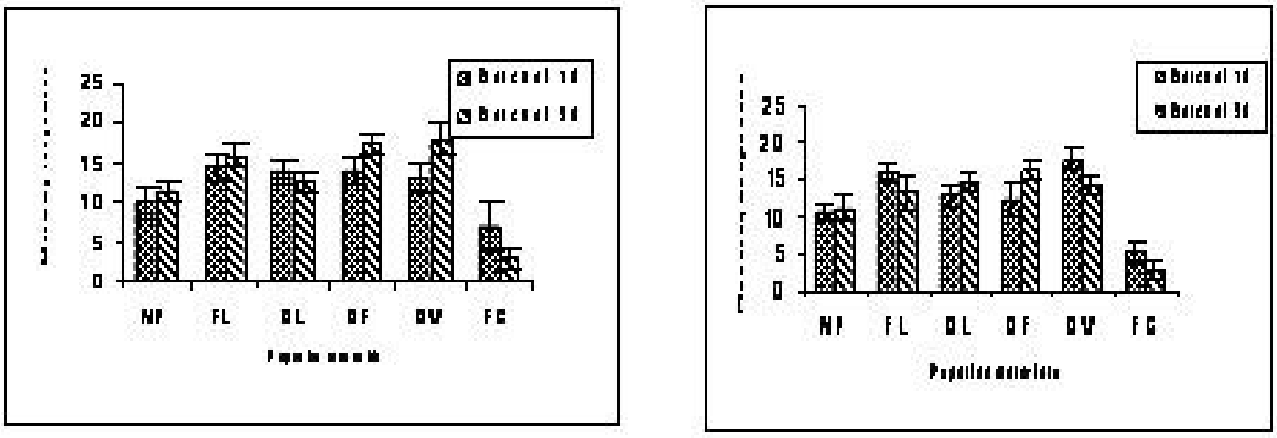
OCT	Batches 10							Batches 50						
P.M	Days 1	2	3	4	5	Total	mean±SD	Days 1	2	3	4	5	Total	mean±SD
N.P.	12	10	11	9	12	54	10.8^{c}±1.300	12	11	10	13	13	59	11.8^{c}±1.303
F.L.	26	7	17	14	16	80	16^{a} ±6.819	22	9	21	14	17	83	16.6^{a}±5.319
D.L.	1	25	14	12	13	65	13^{b}±8.514	3	20	15	13	15	66	13.2b±6.216
D.F.	10	9	17	15	11	62	12.4^{bc}±3.435	15	10	20	17	17	73	14.6^{bc}±3.701
D.V.	26	25	13	13	12	89	17.8^{a}±7.049	20	19	17	15	14	85	17^{a} ±2.549
F.C.	5	7	4	6	4	26	5.2^{d}±1.303	2	4	4	4	6	20	4^{d} ±1.412

News paper strips (N.P), fresh leaves (F.L.), dry leaves (D.L.) decayed fruits (D.F).decayed vegetables (D.V).folded cloths (F.C.); mean values followed by same letters do not differ at ($p \geq 0.01$).

Table- 3 Total number of prepupae in 5 days; (mean±SD) mean value / day and batches of "10" and "50" during the month of December

	Batches 10							Batches 50						
P.M	Days 1	2	3	4	5	Total	Mean±SD	Days 1	2	3	4	5	Total	Mean±SD
N.P.	12	12	8	8	10	50	10^{b}±2.00	12	10	11	13	10	56	11.2^{b}±1.300
F.L.	17	16	15	12	13	73	14.6^{a}±2.07	18	15	17	15	15	80	16^{a}±1.410
D.L.	14	13	14	15	14	70	14^{a}±0.710	11	14	13	12	14	64	12.8^{b}±1.300
D.F.	15	12	13	16	14	70	14^{a}±1.540	19	16	18	16	18	87	17.4^{a}±1.340
D.V.	14	14	14	13	10	65	13^{a}±1.730	16	20	20	18	17	91	18.2^{a}±1.790
F.C.	6	10	7	9	3	35	7c±2.740	4	2	2	3	5	16	3.2c±1.300

News paper strips (N.P), fresh leaves (F.L.), dry leaves (D.L.) decayed fruits (D.F).decayed vegetables (D.V).folded cloths (F.C.); mean values followed by same letters do not differ at ($p \geq 0.01$)



(a) (b)

Fig 1: Responses of *Calliphorid* and *Sarcophagid* flies (mean value per five days) with standard error bars in two batches of "10" and "50" during the months of October (a) and December (b). NF, newspapers; FL, fruit leaves; DL, dry leaves ;DF, decayed fruits; DV decayed vegetables; FC, folded clothes

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Larvae mostly tend to pupariate near the food sources. In the current study, in both the batches larvae liked to find the pupation site near or into the food sources. (Godoy *et al.*,1996). Results showed that decayed vegetables were the best place in both batches in the month of October. In December decayed fruits and fresh leaves were the most preferred site for pupation in the batches of "50" and "10", respectively. In both months and batches folded cloths were proved least attractive material for pupation. In future there is a need of various experiments to determine the effects of temperature, carnivorous pupation, shape of dispersals around the freezing points and photoperiods on the C*alliphorid,* and *Sarcophagide* flies.

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