

OLFACTOMETRY BIOASSAY STUDIES ON BEHAVIOUR OF HOUSE FLIES TOWARDS DIFFERENT FOOD BAITS *IN VITRO*

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ABSTRACT

Food bait preferences of house flies were evaluated using olfactometry bioassays. A single cage dual port glass olfactometer was used to assess preferences of house flies towards sugar, fish meal and molasses in no choice and choice bioassays. Flies were allowed into olfactometer individually, observed for five minutes. Time spent in bait port and decision chamber, number of entries into test/control port and port selected five minutes post-exposure were recorded. In the second study in a four arm acrylic olfactometer, three food baits viz., high fish meal low molasses cake, low fish meal high molasses cake and mango essence were tested. In the glass olfactometer, flies showed positive response towards food baits compared to control in no choice bioassay. In choice bioassay, flies preferred fish meal than both sugar and molasses and molasses was preferred over sugar. In four arm acrylic olfactometer bioassay, 43.87, 30.96 and 25.16 per cent flies were attracted to high fish meal low molasses cake, low fish meal high molasses cake and mango essence, respectively. Results revealed that molasses and fish meal based baits can be used in housefly traps. Olfactometry bioassays were found effective in bait preference studies of house flies.

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INTRODUCTION

Olfaction is the primary sense used by insects to detect and locate various food baits (Whittaker and Feeny, 1971 and Tumlinson *et al.*, 1993). Olfactory cues play a vital role in attracting flies towards traps and stimulate them to go near the odour sources. Any trap system used for housefly, *Musca domestica* necessarily requires a bait to attract them. House flies need protein source to mature sexually and also for the development of their eggs and carbohydrate sources for their energy requirements (Christenson and Foote, 1960). Exploiting this need, housefly traps normally have baits formulated using sugar, protein sources, fermenting sugars and/or a combination of these ingredients. Increased knowledge on behavior associated with attraction of house flies towards baits would help to effectively trap them thereby reducing the fly population (Epsky *et al.*, 1999, Mazor *et al.*, 2002, Smallegange, 2004). One of the effective *in vitro* bioassays that paves the way to find out bait preference of house flies is olfactometry. Here flies are given a choice between baits in a closed controlled environment using olfactometers. Identification and testing of attractant chemical compounds for house flies in addition to toxicity and repellency have been effectively studied using olfactometry (Tang *et al.*, 2016, Tian, 2017, Hung *et al.*, 2015, Khater and Geden, 2019). Two arm and four arm olfactometers play a vital role to find out the bait preferences of house flies so that the most preferred baits or a combination of baits can be incorporated in traps. The present

study was undertaken to evaluate the food bait preferences of house flies using a single cage dual port glass olfactometer using sugar, fish meal and molasses as baits and a four arm acrylic olfactometer where mixed baits prepared from fish meal and molasses were used.

MATERIALS AND METHODS

Maintenance of *M. domestica*

Pupae

Housefly pupae along with litter material were collected from poultry unit at Poultry Research Station, Madhavaram, Chennai. Viable pupae were separated by water floatation method (Welch, 2006). Pupae were then evenly distributed in glass petridishes and fine sand was added (Fig.1). The pupae were kept at room temperature in closed petridishes for emergence of flies. One hundred pupae were kept in each petridish. The petridishes containing pupae were kept in a tray filled with water to avoid predation by ants.

Adult flies

Emerged house flies were transferred to a mini bucket insectary, 27.5 cm height, upper diameter of 22.5 cm and lower diameter of 18.5 cm. The upper surface was covered with a nylon mesh. Handling of fly stages within the insectary was through an opening (10 cm diameter) on lateral lower part which was kept covered with a cloth sleeve secured with clip (Fig. 2). One hundred house flies were kept in the insectary. House flies were given *ad libitum* water. Water was kept in 15 mL plastic

containers provided with a cotton wick (Fig. 3). Flies were fed with honey mixed with wheat bran kept in plastic containers inside the insectary. Food and water were changed every day.

House flies used for behavioural studies were then shifted and maintained in a box type insectary (60 cm L x 45 cm B x 60 cm H) (Fig. 4). Nylon mosquito net was fixed both on lateral sides and rear side whereas the top and bottom portion was closed with wood. Viewing was enabled through transparent glass windows placed on the upper half as well as two windows placed lateral to the outlet which was provided with a sleeve (18 cm L x 15 cm B). All the operations like releasing flies into cage, collection of flies for bioassays, placement of feed and water were carried out through this outlet. The sleeve was tightly closed with a metal clip to prevent escape of flies. Food source for the flies was honey mixed with wheat bran placed in plastic containers. In addition, molasses coated cotton wicks were suspended from the roof of the insectary for flies to feed upon. Predation of flies by ants was prevented by immersing the legs of insectary in plastic petridishes containing water. The insectary was thoroughly cleaned before using it for housefly rearing. House flies were given free access to *ad libitum* water kept in 15 mL plastic containers provided with a cotton wick. House flies were also collected directly from poultry units using a plastic bottle with plunger (Fig. 5).

Preparation of food baits

Sugar, fish meal and molasses were used as food baits. Sugar bait was prepared

by dissolving 4 g granulated sugar in 10 mL of water. Fish meal bait was prepared from Sardine fish (*Sardinella longiceps*). The fish were sun dried, powdered and sieved. Fish meal was prepared at Department of Animal Nutrition, Madras Veterinary College and used for the study. Fish meal bait was prepared by dissolving 4 g of powdered fish meal in 10 mL of water. Sugarcane molasses was obtained from Institute of Animal Nutrition, Post graduate Research Institute in Animal Sciences, Kattupakkam and used directly as bait.

Baits containing fish meal and molasses were also prepared. High fish meal low molasses cake bait was prepared from finely powdered fish meal and diluted molasses (1:3 ratio in water). Ten mL of molasses solution and 90 g of powdered sardine fish meal was taken, mixed well and placed in a 20 cm length plastic mould and dried thoroughly in hot air oven at 60°C till the moisture content falls below 11 per cent. When the mixture turns solid it was removed and stored for use. Low fish meal high molasses cake bait was prepared by following the same procedure for high fish meal low molasses cake except that 20 mL of molasses solution and 80 g of fish meal were used.

***In vitro* olfactometer bioassays for food baits**

Single cage dual port glass olfactometer bioassay

The single cage dual port glass olfactometer was fabricated with clear transparent glass of 3 mm thickness based on

the specifications of Qian *et al.* (2013) with minor modifications. It contained a decision chamber, two test ports and a rear port with a total length of 48 cm. Length, width and height of decision chamber was 96 cm, 50 cm and 25 cm, respectively. Test ports and rear port were 10 cm in diameter and 30 cm in length. The distance between the two test ports was 12 cm. (Fig. 6).

No choice bioassay

This assay involved use of one test bait in one port and control in the other. The study was carried out using three day-old house flies starved for 24 hrs. These house flies emerged from pupae collected from Poultry Research Station, Madhavaram. The food bait to be tested was impregnated in a 2 x 2 cm filter paper (Whatman filter paper No. 1) in one port while in the other port, filter paper impregnated with water was placed as control. Following each trial, the olfactometer was cleaned thrice with 100 per cent methanol prior to reuse. Three food baits viz., sugar, fish meal and molasses were tested. Three day-old house flies from insectary were captured into 15 mL plastic containers individually without damaging them and used for the test. Flies were allowed into the decision chamber of the olfactometer and the entry point was closed with the lid. Each fly was observed for five minutes (Qian *et al.*, 2013). Time spent in bait port and decision chamber was recorded. The number of times fly entered the test/control port as well as the port selected five minutes post-exposure was also recorded. At the end of five minutes the fly was taken out from the olfactometer and discarded. Flies

once used were not reused. Three replicates of the experiment were conducted and in each replicate six flies were used. After each replicate, the olfactometer was thoroughly washed thrice with methanol. Position bias was avoided by changing the ports in which the food bait was placed.

Choice bioassay

The procedure as in No choice bioassay was followed except that comparison between the three food baits viz., sugar, fish meal and molasses was studied (sugar vs. fish meal, fish meal vs. molasses and sugar vs. molasses).

Four arm acrylic olfactometer bioassay

Design of a four arm acrylic olfactometer

A four arm acrylic olfactometer was designed using non absorbent transparent acrylic sheet of 2 mm thickness based on the design of Ranjith (2007) with slight modifications (Fig. 7). The central rectangular chamber was of 20 cm L x 20 cm B x 30 cm H with slots (10 cm square) for insertion of four arms. Four detachable arms made from the non absorbent transparent acrylic sheet of 2 mm thickness each of 50 cm L x 10 cm B x 10 cm H was fixed at the centre of each slot of the central chamber at 5 cm height and inserted into the slot with acrylic rim at connecting end. Each arm was provided with two sliding shutters one at the proximal end and the other at the distal end to control the entry of flies. The top of central chamber was closed with an acrylic lid with an outlet for fly entry and provided with 28 air holes (3 mm diameter). A small axial flow fan (DC 12 V,

3.5 cm dia.) was provided at the end of the distal opening of the arms to provide uniform inwardly directed airflow. The rectangular slot for holding the fan was also provided with a rectangular piece of acrylic leg for the arms to rest.

Choice bioassay

House flies irrespective of age and sex were collected from Poultry Research Station, Madhavaram in plastic containers and brought to the laboratory. Flies were immediately placed inside the insectary and provided with *ad libitum* water and sugar solution. In order to capture active and healthy flies from the insectary, the insectary was covered with black plastic wrap which resulted in the flies moving towards the light towards exit and into the plastic containers. The olfactometer was kept ready with fans on and the shutters at the entry point of four arms were kept closed. Three food baits viz., high fish meal low molasses cake (Fig. 8), low fish meal high molasses cake (Fig. 9) and mango essence (positive control) were taken in a watch glass and kept at the distal end of each arm of the olfactometer. An empty watch glass was placed in the control arm.

Flies were then swiftly released into the central chamber of the olfactometer and allowed to acclimatize for 10 min. After 10 min, all four shutters were opened simultaneously and flies were allowed free access into the four arms of the olfactometer. The olfactometer was left undisturbed for 30 min. At the end of 30 min, the four shutters were closed simultaneously. Number of flies

in each arm as well as the central chamber was counted separately. Flies in the central chamber were considered as non-responders and this number was deducted from the total fly count when the percentage of flies attracted towards individual food baits was calculated. The experiment was repeated five times with new flies. Flies once used were discarded. Position bias was avoided by interchanging the placement of baits in the arms. After each experiment, the olfactometer was cleaned thoroughly thrice with methanol. Percentage of flies responding to each bait was then calculated.

Statistical analysis

The experimental data regarding single cage dual port olfactometry was analyzed by Mann Whitney U test and data on four arm acrylic olfactometry was analyzed by Chi-square test.

RESULTS

***In vitro* Olfactometer bioassay**

Single cage dual port glass olfactometer bioassay

No choice bioassay

Exposure of 18 three days-old 24 hrs starved *M. domestica* flies to fish meal, sugar and molasses baits revealed that 72.22 per cent (13 flies), 72.22 per cent (13 flies) and 88.88 per cent (16 flies) were attracted compared to 0 per cent (no fly), 5.55 per cent (one fly) and 5.5 per cent (one fly), respectively in control. Flies remained in decision chamber were 27.77 per cent (five flies), 22.22 per cent (four flies) and 5.5 per cent (one fly), respectively. Number of

entries made by flies into fish meal, sugar and molasses ports was 41, 26 and 30 with respect to 18, 16 and 17 in the respective control ports. On an average, flies spent 3.5 min, 0.61 min and 1 min, respectively in fish meal port, control port and decision chamber, 2.83 min, 0.94 min and 1.22 min, respectively in sugar port, control port and decision chamber and 3.77 min, 0.77 min and 0.55 min, respectively in molasses port, control port and decision chamber.

Statistical analysis of the observations in no choice bioassay by Mann Whitney U test revealed that the number of entries made by flies into bait ports, fish meal port compared to control port was highly significant (p value: 0.01**^{HS}), entry into sugar port compared to control port was non significant (p value: 0.059^{NS}) while entry into molasses port compared to control port was significant (p value: 0.019*^S). Time spent by flies in bait ports namely, fish meal, sugar and molasses compared to control port was highly significant (p values: 0.000**^{HS}).

Choice bioassay

Comparison of response of flies to sugar and fish meal baits revealed that 61.11 per cent (11 flies) were attracted towards fish meal, 27.77 per cent (five flies) were attracted towards sugar and 11.11 per cent (two flies) remained in the decision chamber. With fish meal and molasses baits, 61.11 per cent (11 flies) were attracted towards fish meal port, 33.33 per cent (six flies) towards molasses port and 5.55 per cent (one fly) remained in the decision chamber. Comparison of sugar

and molasses baits revealed that 66.66 per cent (12 flies) were attracted towards molasses port, 16.66 per cent (three flies) towards sugar port and 16.66 per cent (three flies) remained in the decision chamber. Number of entries made by flies into fish meal port and sugar port were 17 and 11 respectively, fish meal port and molasses port were 22 and 17 respectively and sugar port and molasses port were 23 and 26 respectively. On an average flies spent 1.33 min, 0.77 min and 2.88 min in fish meal port, sugar port and decision chamber respectively. Average time spent was 1.72 min, 1.11 min and 2.16 min in fish meal port, molasses port and decision chamber respectively and 2.16 min, 0.94 min and 1.88 min in molasses port, sugar port and decision chamber respectively.

Statistical analysis by Mann Whitney U test revealed that number of entries made by flies into bait ports, namely, sugar port and fish meal port, fish meal port and molasses port as well as sugar port and molasses port did not show any statistical significance (p values: 0.203^{NS}, 0.372^{NS} and 0.563^{NS}, respectively). Time spent by flies in sugar port and fish meal port as well as time spent by flies in fish meal and molasses ports also did not show statistical significance (p values: 0.462^{NS} and 0.134^{NS}, respectively). Time spent by flies in sugar port compared to molasses bait port showed high statistical significance (p value: 0.008**^{HS}).

Four arm acrylic olfactometer – Choice bioassay

Out of the total 178 house flies used in the study, 87.07 per cent (155 flies) responded to different baits whereas 12.92 per cent (23

flies) did not show any response at the end of the 30 min observation period and remained in the central decision chamber. These flies were considered as non-responders. Out of the 155 house flies that responded to different baits, 43.87 per cent (68 flies) were attracted towards high fish meal low molasses cake, 30.96 per cent (48 flies) towards low fish meal high molasses cake and 25.16 per cent (39 flies) were attracted towards mango essence. No flies were attracted towards the control arm (Table 1). The attraction of flies towards different baits was found to be highly significant statistically when compared to control (χ^2 value = 84.05** $p < 0.01^{HS}$).

DISCUSSION

In chemical ecology research, olfactometry experiments are commonly used to test behavioural preferences in insects by giving a choice between two or more odour sources (Ballhorn and Kautz, 2013). Earlier olfactometry studies by McIndoo (1933) and Brown *et al.* (1961) using house flies revealed that the method was highly efficient to identify specific housefly attractants for use in traps. In the present study, the single cage dual port glass olfactometer fabricated following the design of Posey *et al.* (1998) and Kline *et al.* (2003) was efficient in attracting house flies towards baits as observed by Tang *et al.*, 2016, Tian, 2017, Hung *et al.*, 2015 and Khater and Geden, 2019.

Observations on attraction of houseflies to fish meal powder in this study are in consistent with Burg and Axtell (1984), Pickens *et al.* (1986) and Zhen *et al.*

(2004). In the bioassay using sugar baits, house flies exhibited increased preference in terms of selection of ports, number of entries and time spent in the port as against control as recorded by Pickens *et al.* (1994a); Hogsette *et al.*, (2002) and Khan *et al.* (2013). Attraction of house flies towards molasses based baits corroborated with that of Carlson and Hogsette (2007) and Albarrak (2009). In choice bioassay, the preference of fish meal over sugar was highly evident which may be presumably due to distinctive volatile profile relative to the manure-associated odours found in the premises of livestock and poultry units (Qian *et al.*, 2013). Based on our observations in choice bioassay, fish meal based baits was found the most preferred followed by molasses and sugar the least among the three.

In earlier studies (Landoldt, 1995; Geden *et al.*, 2006 and 2009), molasses was observed to be significantly more attractive to house flies than honey, maple syrup or jaggery which agrees with the findings recorded in the present study. Between fish meal and sugar, increased attraction was observed towards fish meal than sugar (Warner, 1991) and fish meal based baits were found to be highly effective in attracting house flies even in the presence of competing food odours in outdoor stations (Geden, 2005).

In the present study, 24 hrs starved house flies showed more preference towards molasses than sugar as observed by Albarrak in 2009. In another field study undertaken by Geden *et al.* (2008), 25 per cent dilution of blackstrap molasses in cattle feed was found

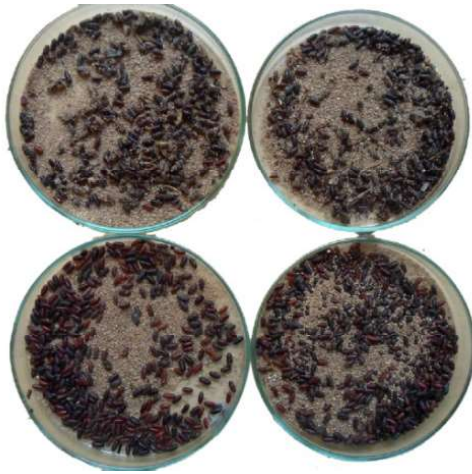


Fig 1. Pupae of housefly collected from poultry shed kept in sand for emergence of house flies



Fig 3. Plastic containers with cotton wick filled with water for house flies



Fig 2. Mini bucket insectary for rearing house flies



Fig 4. Box type insectary for rearing house flies

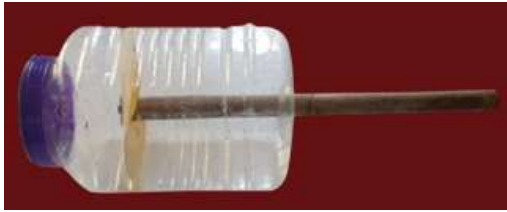


Fig 5. Plastic bottle with plunger for collecting house flies from poultry unit



Fig 6. Single cage dual port glass olfactometer for testing food bait preference in house flies

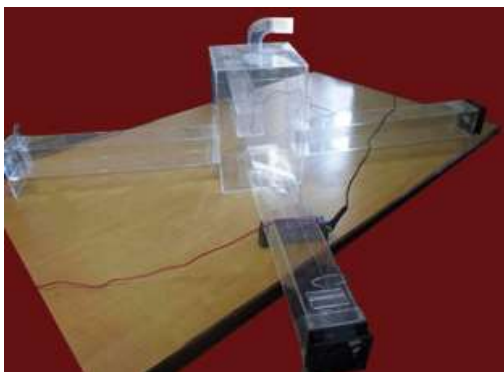


Fig 7. Four arm acrylic olfactometer for testing food bait preference in house flies



Fig 8. High fish meal low molasses cake

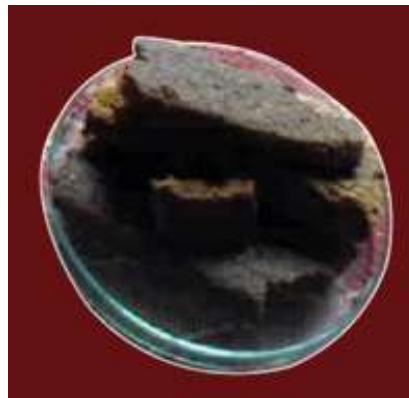


Fig 9. Low fish meal high molasses

to be equally effective as the commercial attractant used.

It is opined that flies learn to correlate their previous experience with the presented baits to choose the appropriate one (Zrelec *et al.*, 2013). According to Ballhorn and Kautz (2013), the variation in age and sex of the flies influence the responds towards baits in olfactometer. They also stated that the age

Table 1. Housefly response to different food baits in choice bioassay**(n=178 house flies of mixed sex)**

Replicate	No of flies tested	House flies attracted in 30 min									
		Mango Essence (Positive control)		High fish meal low molasses cake		Low fish meal high molasses cake		Control		Non-Responders (NR)	
		No of flies	per cent	No of flies	per cent	No of flies	per cent	No of flies	per cent	No of flies	per cent
1.	38	7	18.42	15	39.47	10	26.31	0	0	6	15.78
2.	40	10	25.00	13	32.50	12	30.00	0	0	5	12.5
3.	35	8	22.85	14	40.00	9	25.71	0	0	4	11.42
4.	38	11	28.94	16	42.10	8	21.05	0	0	3	7.89
5.	27	3	11.11	10	37.03	9	33.33	0	0	5	18.51
Total	178	39	21.91	68	38.20	48	26.96	0	0	23	12.92
Total after deducting NR	155	39	25.16	68	43.87	48	30.96	0	0		

χ^2 value = 84.05** ($p < 0.01$)^{HS}(excluding non-responders)

of insects plays a major role in the decision making ability. Older insects which are more experienced have been reported to respond more carefully (Ballhorn and Kautz, 2013). In the present study, newly emerged flies subjected to olfactometry on the first day of emergence did not show any responses because most of the flies were found in the decision chamber without entering into the baited ports and similar observations were recorded by Murvosh *et al.* (1965) and Ballhorn and Kautz (2013).

Four-arm olfactory chambers provide free movement for flies (Vet *et al.*, 1983 and Bakchine *et al.*, 1990) and have been successfully used by entomologists to study food bait preference in insects such as *Trichogramma chilonis* Ishii, the egg parasitoid of the rice leaf folder, *Cnaphalocrocis medinalis* (Ranjith, 2007), oil palm pollinating weevil, *Elaeidobius kamerunius* (Adaigbe *et al.*, 2011), melon fly, *Bacterocera cucurbita* (Bothon *et al.*, 2013) and weevil, *Aegorhinus superciliosus* (Espinoza *et al.*, 2016).

In the present study, house flies showed increased preference to high fish meal low molasses cake followed by low fish meal high molasses cake and mango essence compared to control which indicates that flies showed more preference towards combination baits (bait cakes) made of fish meal and molasses than single baits (mango essence). Superiority of combination of baits over single baits have been proved by Brown *et al.* (1961), Mulla *et al.* (1977), Qian *et al.* (2013), Smallegange (2004), Cosse and Baker (1996) and Brown *et al.* (1961).

Natural substances which may serve as oviposition substrates and/or food sources have been shown to be attractants for house flies especially putrefying and fermenting protein substrates like fish meal emanating amines, aldehydes, ketones and alcohols (Brown *et al.*, 1961; Kunast and Gunzrodt, 1981 and Cosse and Baker, 1996). This was observed in the present study where fish meal baits containing putrefying components and molasses with fermenting substances attracted more flies as compared to single bait containing mango essence.

The cake baits prepared in the current study were also less odorous unlike other housefly attractant baits that contain fetid odorants which makes it even more acceptable for use in indoor especially in locations where other fly lures cannot be used.

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