Full Length Article

DODECAGON MAZE BIOASSAY TO ASSESS COLOUR BAIT PREFERENCES OF HOUSE FLIES

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ABSTRACT

Colour bait preferences of Musca domestica (house fly) was evaluated in a dodecagon maze. Flies collected from a poultry unit maintained in an in sectary were used for the study. An acrylic dodecagon maze was fabricated with dimensions 30 cm L x 25.5 cm B x 26 cm H with a lid. Twelve chambers were colour coded with red, black, blue, green, yellow and white in duplicate. House flies were swiftly released into the central chamber of dodecagon maze in batches, lid closed, observed for 30 min. Out of the total 718 house flies evaluated, 81.05 per cent (582 flies) responded to different colours whereas 5.01 per cent (36 flies) were non responders. Out of the 582 house flies responded to different colours, 30.41 per cent (177 flies), 21.99 per cent (128 flies), 16.32 per cent (95 flies), 14.43 per cent (84 flies), 10.48 per cent (61 flies) and 6.35 per cent (37 flies) preferred red, black, green, yellow, blue and white colours, respectively. Preference of house flies towards different colours compared to white was found to be highly significant statistically. Findings of the present study indicate that red being the most preferred colour, it can be used in house fly traps.

Keywords: House fly, *Musca domestica,* Colour bait preference, Dodecagon maze, Bioassay.

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INTRODUCTION

One of the major factors that affect behavior of flies towards targets or traps is visual stimuli (Zuker, 1995). Colours are potential visual cues to trap insect pests. House flies are attracted to colours and therefore fabrication of traps with colours preferred by them is vital in achieving maximum trap efficiency (Geden *et al.,* 2006; Diclaro *et al.,* 2012).

Optical sensitivity of housefly lies between 310 nm and 700 nm (Strother and Casella, 1972) and this sensitivity vary according to factors such as presence of vegetation, sex of flies and other environmental factors (Veal *et al*., 1995). Before designing a housefly trap, it is therefore essential to assess the colour preference of house flies by exposing flies to different colours of various wavelengths using *in vitro* bioassays. However, house flies have a visual sensitivity peak in the 470-520 nm range and sensitivity in this region may help flies to locate non-green objects in an environment dominated by vegetation (Veal *et al*., 1995). According to Hardie (1986), photoreceptors of the compound eye of house flies have three absorbance peaks including one at 490 nm (blue/green) and a second at 570 nm (yellow) and a third which lies within the UV range. Colour is one of the potential cues that trigger aggregation of house flies. It is widely believed that variation in trap colour significantly enhances catch rates of house flies (Chapman *et al.,* 1999). According to Hanley *et al*. (2009), a clear sexual dichromatism is found in house flies. Assessment of colour bait preferences in house flies can be effectively done in a dodecagon maze. Dodecagon is a polygon that has twelve sides. Dodecagon maze is a device commonly used in behavioural studies of experimental animals such as rats. This device is also used to test colour preferences of flies to multiple colours simultaneously. It was used to find out the colour preferences of the oriental fruit fly, *Bactrocera dorsalis* (Wu *et al*., 1993). Wang *et al.* (2019) also used the dodecagon maze to study the visual responses of the fruit fly, *Bactrocera minax*. Housefly responses to different colours in a dodecagon maze have never been investigated. Hence, the present study was undertaken to test the preferences of house flies to different colours, viz., red, black, blue, green, yellow and white in a dodecagon maze.

MATERIALS AND METHODS

Collection and maintenance of *Musca domestica*

Flies were collected from the floor of units housing chicken in M-type battery cages at Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai. Flies were lured by placing a drop of liquid molasses as bait in a thick sheet of paper at floor level in active breeding areas. Flies that gathered around the molasses bait were collected using a plastic container with a plunger and were then carefully shifted to aerated containers without causing any damage to flies. Collected flies were then transported to the laboratory at the Department of Veterinary Parasitology, Madras Veterinary College, Chennai. House flies were identified based on gross morphological characters observed using a hand lens. After identification, house flies were transferred and maintained in a box type insectary of dimensions 60 cm L x 45 cm B x 60 cm H (Fig. 1). Mosquito net of 1.2 mm mesh size was affixed with nails both on lateral sides and rear side whereas the top and bottom portion was closed with plywood. Viewing was enabled through transparent square areas of glass made on the upper half of the front side of the insectary. To gain access to the insectary for operations such as releasing flies into cage, a square outlet with a sleeve was provided. The sleeve was tightly closed with a metal clip

to prevent escape of flies. Collection of flies for bioassays, placement of feed and water and cleaning of the insectary were carried out through this outlet.

Food source for the flies was honey (Dabur®) mixed with powdered wheat bran placed in rectangular plastic 60 ml containers at the floor of the insectary. In addition, liquid sugarcane molasses coated cotton wicks (10 cm length) were suspended from the ceiling of the inner middle portion of the insectary as food source for flies. Entry of predatary ants and other insects into the insectary was prevented by placing the legs of the insectary in petridishes containing water. House flies were given *ad libitum* water filled into 15 ml plastic containers provided with a cotton wick, placed on the floor of the insectary. The insectary was thoroughly cleaned with a dry cloth before accommodating a new batch of flies.

Design of the dodecagon maze

The acrylic dodecagon maze was designed as per the specifications of Wu *et al*. (1993, 2007). The maze was fabricated using transparent acrylic of 2 mm thickness with an outer diameter of 101 cm. There were twelve chambers each with a dimension of 30 cm L x 25.5 cm B x 26 cm H. The diameter of the middle circular decision-making chamber area was 50 cm. The colour sheets were pasted on the inner and outer walls of the chambers of the maze. The maze was closed with the lid (Fig. 2).

Colour coding of the dodecagon maze

Six colours viz., red, green, blue, yellow, white and black were tested for the preference by house flies in the dodecagon maze. The wavelengths of red, green, blue and yellow colours used in the study when assessed by comparison with colour wavelength chart were 625-740 nm, 500-565 nm, 440-485 nm and 565-590 nm, respectively.

***In vitro* testing of colour preferences**

The testing was done based on the protocol of Wu *et al.* (1993) with minor modifications. House flies were collected from the insectary through the sleeve in plastic aerated containers. Flies were forced to move near the natural light by closing the entire insectary with a black coloured sheet. Flies (males and females together) in batches of 140-150 numbers were swiftly released from the aerated containers directly into the central chamber of the dodecagon maze by opening the lid of the maze. Flies released in the central decision-making chamber were able to see all the colored chambers simultaneously for decision making. Once all flies of a batch were released into the maze, the lid was closed. The maze was left undisturbed for 30 min on a wooden table in a well illuminated laboratory. At the end of 30 min, number of flies resting in each colour coded chamber was counted. Flies in the central chamber were also counted separately and these flies were considered as non responders and this number was deducted from the total fly count when the percentage of flies attracted towards individual colours was calculated. Experiment was repeated five times with new flies and flies once used were discarded. Flies were allowed to remain in the maze until they die due to exhaustion and starvation, collected and disposed. After each replicate, the dodecagon maze was cleaned

thoroughly with cotton soaked in water. The experiment was conducted in a block design with each fly serving as a block being exposed to all colours in the dodecagon maze.

Statistical analysis

Data were analyzed by Chi-square test using Microsoft Excel and IBM™ SPSS™ Version 20.0 for Windows™.

RESULTS

Out of the total 718 house flies tested for colour preference in the dodecagon maze, 81.05 per cent (582 flies) responded to different colours, whereas 5.01 per cent (36 flies) did not show any response at the end of the 30 min observation period and remained in the central decision chamber. Red colour was observed to be the most preferred colour by house flies followed by black, green, yellow, and blue whereas white was least preferred. Out of the 582 house flies which responded to different colours, 30.41 per cent (177 flies), 21.99 per cent (128 flies), 16.32 per cent (95 flies), 14.43 per cent (84 flies), 10.48 per cent (61 flies) and 6.35 per cent (37 flies) were attracted to red, black, green, yellow, blue and white colours, respectively. The preference of flies towards different colours compared to white colour was found to be highly significant statistically (χ^2 value = 153.77** $p<0.01^{HS}$). Statistical significance was also found in each replicate (Table 1, Fig.3).

DISCUSSION

Visual stimulation is one of the major determinants in housefly behaviour which influence strongly either as attraction or repulsion towards baited targets (Zuker, 1995, Geden *et al.,* 2006) and colours improve fly trap performance (Diclaro *et al.,* 2012). Despite many experiments, there remains a great deal of uncertainty about colour preference in house flies, often contradictory results emerge from studies conducted in indoor and outdoor units (Hanley *et al.,* 2009 and Howard and Wall, **(**1998).

In the present study, house flies showed preference to red and black compared to blue and white as observed by Waterhouse (1948), Pospisil (1962) and Hecht (1963). Welch (2006) reported that yellow, green and white colours were more attractive for flies than black colour and Waterhouse (1948) claimed that white and blue were the least preferred as compared to green, yellow, blue, and red. Findings of the present study concerning attraction towards black colour are not in accordance with Welch (2006) but the least preference to white is similar to the observations of Waterhouse (1948) and Hecht *et al.* (1968). More attraction towards yellow compared to white is in accordance with earlier reports (Burg and Axtell, 1984; Pickens and Mills, 1993). Ahmed *et al.* (2005) reported that sugar coated black and green strips attracted more house flies than other colour strips and white strips being the least preferred as recorded in the present study.

Nihad (2011) and Otto *et al.* (1967) observed that black colour traps caught the maximum number of flies followed by green, yellow, white which is also in accordance with the present study. Ryu *et al.* (2014) concluded that the most and least preferred colours were blue and red, respectively, which

Table 1. Colour preferences of house flies in Dodecagon maze

Replicate	Total no of flies tested	Colour preference of house flies						χ^2 value
		Red	Black	Green	Yellow	Blue	White	
1	122	41	30	23	16	9	3	57.56**
2	135	40	31	16	22	12	14	32.19**
3	152	47	34	22	19	18	12	39.19**
4	107	30	23	19	17	12	6	23.61**
5	66	19	10	15	10	10	2	17.89**
Total	582	177	128	95	84	61	37	

**-P<0.01

is contradictory to the present findings and also to other earlier published reports except Welch (2006). One of the reasons for lowest preference of house flies towards white could be the wide range of wavelengths in white colour which may create a conflicting stimulus to flies (Diclaro *et al.,* 2012) in addition to its broad spectral reflectance (Hanley *et al.* 2009). Weather conditions such as air temperature may also influence the attraction of house flies to specific colours (Muniz, 1967 and Pickens, 1995). More attraction of flies towards black colour could be attributed to the darker nature of harborage sites or resting sites of flies and also this colour could add stimulus to satisfy the scototactic tendency of house flies (Hecht, 1970). A similar dodecagon maze study by Wu *et al.* (1993, 2007) to test the color preference of the fruit fly *Bactrocera dorsalis* revealed that green, yellow and orange colours were found to be significantly more attractive than red, black and white. White colour was found to be less attractive than red and black. However, house flies are attracted to white surfaces that give off odours (Sanchez-Arroyo and John L. Capinerva, 2017). For instance chicken eggs are milky white in colour and have more than 7000 pores that allow carbon dioxide to escape out and fresh air to get in. Eggs also have odours due to dietary factors which release trimethylamine. These substances attract flies to chicken eggs and hence fly specks are commonly noticed on the surface of chicken eggs. Spot cards which are white index cards are generally recommended as an easy-to-use method for monitoring house fly density and activity. Since *Musca domestica* rests on surfaces, the spot card was utilized to quantitatively study the intensity of infestation. The white background of the spot card was mainly to enable easy visualization of the brown coloured specks.

Figure 1: Box type insectary for maintenance of house flies

Figure 2: Acrylic dodecagon maze for testing colour preference in house flies

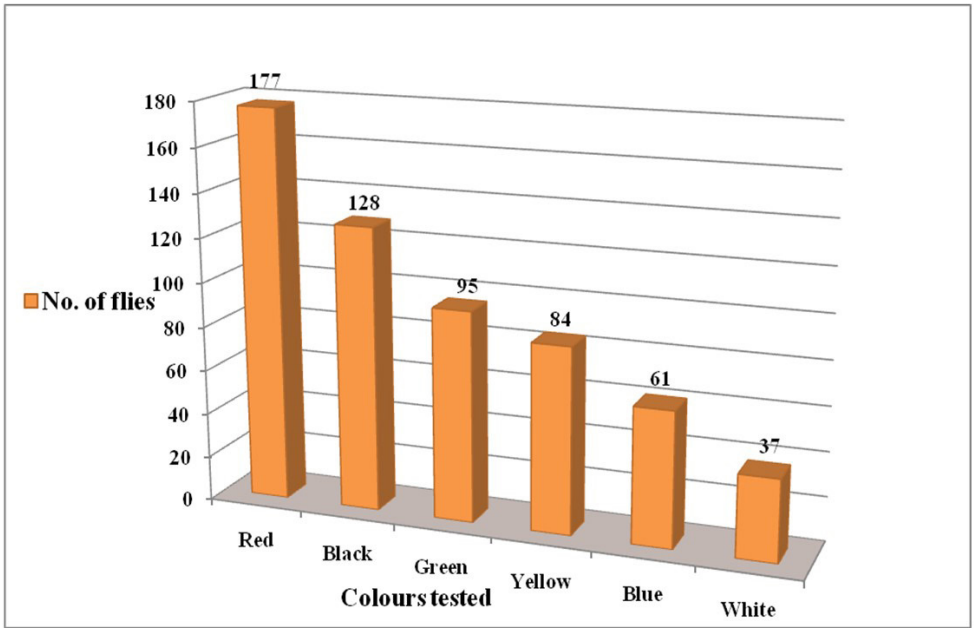

Figure 3: Colour preference by house flies in the Dodecagon maze bioassay

χ^2 value = 153.77** ($p<0.01$)^{HS} (excluding non-responders)

This is the first study where six colours were simultaneously tested for the preference of house flies in a dodecagon maze and based on the observations, red colour was found to be the most alluring colour to house flies and hence this colour can be preferred in fabrication of housefly traps. Flies were given 30 minutes time to make active choice of the colours without following the predecessors. However, detailed field study using different coloured traps need to be carried out to find out whether there is any contradiction in the preference of colours by house flies in field conditions compared to laboratory bioassays. Variation in colour preferences among different strains of house flies and the influence of other environmental parameters also need to be studied.

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