Full Length Article

CHEMOTACTOMETER AND GLUE TRAP BIOASSAYS TO EVALUATE PELLET BAITS TO LURE HOUSE FLIES

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ABSTRACT

Different pellet baits were prepared and evaluated for their efficacy to lure house flies in chemotactometer and glue trap bioassays. Pellets with fish meal alone (FM), fish meal + (Z)-9-tricosene (FMP) and fish meal + (Z)-9-tricosene + butylated hydroxyl toluene (FMPB) were prepared. In the chemotactometer bioassay, house flies comprising of a mixed population of males and females were released into the four arm acrylic chemotactometer, acclimatized for 10 min and observed for 30 min. Out of the 201 house flies tested in six replicates, 65.17% (131 flies) responded to the baits. The attraction was maximum towards FMP pellets (49.61%) followed by FMPB (25.19%) and FM pellets (8.39%) with least preference towards the unbaited control (11 flies, 8.39%). The per cent fly activity was 64.82%. Flies attracted towards FMP and FMPB pellets preferred to stay and spend more time inside the baited arms of the chemotactometer exhibiting wing fanning and mating behaviour. In the glue trap bioassay, a glue trap was fabricated in thermacol sheet of 20 cm L x 10 cm B x 3 cm H with a red glue sheet pasted on it and baited with pellet baits. The traps were then placed on the floor of the insectary containing house flies for 24 h. Out of 310 house flies trapped, maximum attraction was elicited towards FM pellets (29.35%, 91 flies) followed by FMPB (27.09%, 84 flies) and FMP pellets (25.48%, 79 flies) with least attraction towards unbaited control traps (18.06%, 56 flies).

Results revealed that in the chemotactometer bioassay, more flies were attracted towards the pellet baits with (Z)-9-tricosene (FMP) followed by FMPB and FM pellets.

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In the glue trap bioassay, attraction was more towards FM pellets followed by FMPB and FMP pellets. Overall, male flies were found more attracted towards FMP and FMPB pellets. Both the bioassays were found efficient to evaluate the attractiveness of pellet baits for house flies and (Z)-9-tricosene incorporated in fish meal and molasses as pellet baits can be effectively used in house fly traps to improve trap efficiency.

Keywords: housefly, olfactometry, fish meal bait, chemotactometer bioassay, glue tap bioassay

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INTRODUCTION

House flies (*Musca domestica L.*) are pervasive pests, accompanying humans worldwide and serving as nuisance and vectors of pathogens. Their biology and behavior equip them for successful survival, yielding large persistent populations that subvert every control method directed against them (Hinkle and Hogsette, 2021). These flies are potential transmitters of bacterial, viral and parasitic pathogens to human beings and animals. An integrated approach is vital to effective control of synanthropic pests. House fly traps play an important role in integrated fly management practices. Use of pheromone-based baits in traps to attract and kill house flies considerably reduces the need of chemicals and is ecosensitive. Pheromones are chemicals produced and used by insects to lure other members of the same species. The best known are sex pheromones which are emitted usually by the females of many species of insects helps to indicate their location and willingness to mate. Musca domestica sex pheromones have been studied in detail by researchers but only one, (Z)-9-tricosene known commercially as "muscalure" appears to have been successfully demonstrated for field use (Carlson *et al.,* 1971). It is a component of the cuticular hydrocarbons (waxes) and faeces of unmated female house flies and was found to be attractive to male houseflies in laboratory experiments and in field trials (Butler *et al.,* 2007, Butler, 2007). In fact (Z)-9-tricosene seems to act as a chemical confirmation to the male that the female that it has encountered is indeed a M. domestica and not some other species. (Z)-9-tricosene acts as an attractant or aggregation pheromone and being a highly volatile compound can only influence the behavior of house flies at short ranges (Noorman and den Otter, 2001; Kelling *et al.,* 2003). Pheromones when combined with other food attractants and essential oils can be effectively used to lure house flies (Khater and Geden, 2019). One of the major problems associated with the use of (Z)-9-tricosene is its volatility and biodegradability. (Z)-9-tricosene based pheromone baits for flies do not last long unless they are impregnated into sustained release or slow release dispensers (Chapman *et al.,* 1998a; Hummel *et al.,* 2013). Even though pheromone based fly control is a viable practice, suitable pheromone dispensers, however are wanting. Baits with pheromone combined with food lures usually have longevity and sustainability. Chemotactometers are used effectively in chemical ecology research and are highly useful to evaluate bait preferences

by flies (Adjavon *et al.,* 2021; Tang *et al.,* 2016). In the present study different pellet baits based on pheromone and food lures were prepared using fish meal, molasses, (Z)-9-tricosene and evaluated for their attraction to house flies in vitro in chemotactometer and glue trap bioassays.

MATERIALS AND METHODS

Preparation of pellet baits

The house fly sex attractant pheromone (Z)-9-tricosene (97%) was purchased from Sigma Aldrich, Germany. Sardine fish meal was used to impregnate (Z)-9-tricosene and made into pellets. Pellets were prepared at Central Institute for Brackish water aquaculture, Santhome, Chennai (CIBA). (Z)-9-tricosene was dissolved in hexane and 0.22 per cent w/v was added to fish meal and mixed thoroughly. Undiluted molasses was added to this at 5 per cent level as a binder as well as a food lure in all the three pellet baits and the mixture was evenly mixed and stuffed into a tablet press (customized at CIBA) and pellets were prepared. Pellets with antioxidant butylated hydroxy toluene (BHT) at 0.22 per cent w/v were also prepared to study the efficacy of the antioxidant in sustained release of the pheromone (Table 1). Fish meal base of 100 g yielded 32 pellets. Each pellet had a diameter of 10 mm and length of 20 mm with an average weight of 3.13 g. Pellets with fish meal alone, fish meal and (Z)-9-tricosene and fish meal, (Z)-9-tricosene and BHT were named FM, FMP and FMPB pellets, respectively.

Collection of house flies

House flies were collected from poultry units housing layer chicken in "M" type battery cage system of rearing from Poultry Research Station, Madhavaram in plastic containers and used for the study. Pin holes were provided on the cap of the containers to provide sufficient aeration for the flies during transit.

Chemotactometer bioassay for pellet baits

A four arm acrylic chemotactometer was used. It was designed using non absorbent transparent acrylic sheet of 2 mm thickness based on the design of Ranjith (2007) with slight modifications (Fig 1). 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 detachable arms. The arms made from 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 a height of 5 cm. 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.

In the pheromone attraction study, all the three types of pellets (FM, FMP and FMPB) were used. Two pellets were kept in a watch glass which was then placed at

the middle of each arm. One arm was used as unbaited control with empty watch glass without any of the pellet baits. A total of 201 randomly selected flies comprising of mixture of both males and females in six replicates were utilized for the present study. Flies were swiftly released into the central chamber of the chemotactometer and allowed to acclimatize for 10 min. Acclimatization was done in order to facilitate the flies to adapt to the ambient conditions before testing. After 10 min, all four shutters were opened at the same time. The chemotactometer was left undisturbed for 30 min. At the end of 30 min, the four shutters at the proximal end of arms 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 baits was calculated. Experiment was replicated six times with new flies. Flies once used were discarded. Position bias was avoided by rotating the bait arms between each replicate. After each experiment the chemotactometer was cleaned thoroughly thrice with hexane. The same pellets were used throughout the study. Percentage of flies responding to each pellet bait was then calculated to arrive at the order of preference of pellets. Sexing of flies attracted towards various baited pellets was also carried out based on holoptic eyes in males and dichoptic eyes in females. Activity and behavior of flies in the chemotactometer was also monitored. Percentage fly activity and percentage fly preference was calculated using the following formulae.

$$\text{Percentage fly activity} = \frac{\text{No. of flies released in the chemotactometer – non responding flies}}{\text{Total number of flies released}} \times 100$$

$$\text{Percentage fly preference} = \frac{\text{Flies attracted towards test arms – Flies attracted towards control}}{\text{Total number of flies released – non responding flies}} \times 100$$

Glue trap bioassay for pellet baits

Comparative efficacy of pellet baits in luring house flies was also evaluated using glue traps (Fig. 2). Thermacol of 20 cm L x 10 cm B x 3 cm H was taken to which was affixed red colour glue sheets on one surface. Four such traps were prepared. Three of them were baited with two pellets each (FM, FMP and FMPB). The pellet baits were placed at the middle of the trap. One unbaited trap was used as control. The glue traps were randomly placed inside the insectary containing house flies and left undisturbed for 24 h. Then the traps were taken out, number of house flies lured and got trapped were counted and sexed. The experiment was repeated six times and percentage of house flies attracted towards the different pellet baits was analyzed. Position bias was avoided by changing the placement of the baited and control traps inside the insectary for each replicate.

Statistical Analysis

Data analysis was carried out by Chi-square test using SPSS 20 version software (SPSS 20.0; Inc., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Insects emit volatile chemicals called pheromones to lure members of the same species. Most of the female flies produce sex pheromones to attract males which can be effectively used as baits to lure flies towards traps. Chemotactometers are used effectively in chemical ecology research and are highly useful to evaluate bait preferences by flies. In the present study, three different pellet baits were prepared and evaluated in chemotactometer and glue trap bioassays.

Chemotactometer bioassay for pellet baits

Out of the 201 house flies tested in six replicates, only 65.17 per cent (131 flies) responded to the baits whereas the remaining 34.82 per cent (70 flies) showed no response and remained in the central decision chamber (non-responders). Among the 131 flies responded, the FMP pellet attracted the most number of flies (65 flies, 49.61%) followed by FMPB pellet (33 flies, 25.19), FM pellet (22 flies, 16.79%) and the least number of flies were attracted towards control arm (11 flies, 8.39%). The preference of flies towards the pellet baits was found to be highly significant statistically ($\chi 2$ value = 70.70** $p<0.01$HS) when compared to control. The fly activity was 64.82%. Fly preference towards FM, FMP and FMPB pellet baits was 10.07%, 43.41% and 18.60%, respectively. Those flies which were attracted to FMP and FMPB pellets also showed characteristic behavioural changes such as wing fanning, high level of activity, frequent landings on the pellets and exhibition of mating behavior. Flies attracted towards the non-pheromone pellets were found to be static most of the times.

Sex variation was observed in flies lured towards the three different pellet baits. FM pellet attracted a greater number of female flies than males (19 female flies, 86.36% vs 3 male flies, 13.63%) whereas a higher number of male flies were attracted towards both FMP pellet (52 male flies, 80% vs 13 female flies, 20%) and FMPB pellet (29 male flies, 87.87% vs 4 female flies, 12.12%). In the unbaited control arm, 6 out of 11 (54.54%) flies were males and remaining five (45.45%) were females. Variation in male and female flies within baits was found to be statistically significant ($\chi 2$ value = 85.77** $p<0.01$HS for male flies and 74.69** $p<0.01$HS for female flies).

Flies attracted towards FMP and FMPB were predominantly males proving the fact that Z-(9)-tricosene specifically attract male flies when exposed to the pheromone source in a closed, confined and controlled environment of chemotactometer. The increased attraction of male house flies to Z-(9)-tricosene in chemotactometer was in agreement with the findings of Carlson *et al.* (1971). Further, Carlson and Beroza (1973) observed that only male house flies were attracted towards (Z)-9-tricosene impregnated filter paper kept inside a chemotactometer. In the present study, though male flies were predominant, female flies were also attracted towards the pellet

baits. This could be due to the presence of fish meal and molasses in the pellet baits which are preferred food choices for house flies. Use of food lures along with pheromones as baits thereby synergistically attracts both male and female flies and could be effective for use in traps.

Behavioural changes in house flies exposed to (Z)-9-tricosene has also been well documented. Sexual stimulation in house flies especially males exposed to (Z)-9-tricosene such as mating strike activity and copulatory activity have been observed in chemotactometer studies by Carlson *et al.* (1971) and Adams and Holt (1987). In the present study also, behavioral changes were observed in house flies that selected the arms of chemotactometer baited with FMP and FMPB pellets which had impregnated (Z)-9-tricosene. These findings were in agreement with the observations made by the above authors. Flies also spent more time near the pheromone pellet baits as observed by Adams and Holt (1987) who observed the arrestment effect of (Z)-9-tricosene in addition to its function as a short range sex attractant as well as sex recognition factor. Studies conducted by Rogoff *et al.* (1964) revealed that male house flies positively responded to (Z)-9-tricosene with attempts of copulatory strikes when it was impregnated in a knot of black thread about the size of house fly which was called "pseudo fly". Similar observations were made in the present study on behavior of male house flies towards the pheromone impregnated pellet baits.

Glue trap bioassay for pellet baits

Out of the 310 house flies trapped, maximum of 29.35% (91 flies) were trapped in the glue trap with FM pellet followed by the trap baited with FMPB pellet which attracted 27.09% (84 flies) while the trap baited with FMP pellet attracted only 25.48% (79 flies). The control trap attracted the least number of flies (18.06%, 56 flies). The variation in the number of flies lured to different pellet baits was significant statistically ($\chi 2$ value = 11.89** $p<0.05S$). Among the 310 flies trapped, 71.33 per cent (222 flies) were males and 28.66 per cent (88 flies) were females. In FM pellet baited trap, out of 91 flies trapped, 59.34 per cent (54 flies) were males and 40.65 per cent (37 flies) were females. In FMP pellet baited trap, out of 79 flies, 79.74 per cent (63 flies) were males and 20.25 per cent (16 flies) were females. In FMPB pellet baited trap, out of 84 flies, 86.90 per cent (73 flies) were males and 13.09 per cent (11 flies) were females. In control trap, out of 56 flies, 51.78 per cent (29 flies) were males and 48.21 per cent (27 flies) were females.

Comparison of the effectiveness of the fish meal pellet dispensers in glue trap bioassay revealed that more flies were lured to the FM pellet (29.64 per cent) followed by FMPB pellet (27.36 per cent) and FMP pellet (25.73 per cent) although the difference in number of flies was not significant. Unbaited control traps could lure only 18.24 per cent of flies.

According to the expectations, overall trap catches were dominated by male flies. There was a vast variation in the sex of *M.*

domestica trapped where 71.33 per cent constituted male flies and females were only 28.66 per cent. The attraction of more male flies towards Z-(9)-tricosene was evident in both FMP and FMPB pellets with a ratio of 6:1 and 7:1, respectively. In FM pellet baited traps and control traps as well, the males were higher with a ratio of 3:2 and 5:4, respectively. The increased number of male flies indicates that Z-(9)-tricosene is effectively impregnated in fish meal pellet baits and able to effectively lure the flies. The increased number of male flies in FM baited trap could also be attributed to the closeness of the FM pellet baited trap to the FMP and FMPB pellet baited traps inside the insectary which could have influenced the odour plume in the microenvironment attracting more males.

In vitro laboratory studies have demonstrated that Z-(9)-tricosene is highly attractive to male house flies (Silhacek *et al.,* 1972; Nicholas, 1988) and findings in the glue trap bioassay were in accordance with these observations. Attraction of a greater number of male house flies towards (Z)-9-tricosene in the present study matches with the findings of Carlson *et al.* (1971) and Carlson and Beroza, (1973). In the current study the number of fly visitations towards the pellet baits was more compared to the number of flies that actually got trapped. More number of flies was found to escape from getting trapped in the glue and more flies were found to feed on the pellet baits after landing on the pellets and flies were found to spend more time on and around the pellets. The feeding activity of flies on pellet baits would have made them wet thereby increasing the moisture content of the fish meal base and exposure of the impregnated (Z)-9-tricosene which in turn could have attracted more male flies towards the pellets. This interesting finding is supported by the observations of Barnhart and Chadwick (1953) who postulated the existence of an unknown substance termed "fly factor" in *M. domestica* i.e. the food that was fed on by flies became more attractive to other flies thereby attracting them towards the baits. Acree et al. (1959) also showed in a series of experiments that increased attractiveness of sugar that had been fed on by house flies was probably due to the increase in the moisture content or the microclimate prevailing in the pellet baits which favours further attraction of additional flies. In addition, since that the glue trap assay was conducted using field collected flies and flies could have been an admixture of hungry flies, fed flies, newly emerged flies and flies of different age groups and physiological status and presence of fish meal and molasses in the pellets which act as phagostimulant, could have played a role in luring flies since house flies have the tendency to congregate in areas of available food sources (Murvosh *et al.,* 1965). Present studies endorse these reports. Further, detailed studies are needed to assess the attraction of fish meal pheromone pellet baits in vitro to house flies pertaining to differences in age, sex, physiological status and breeding habitats.

Table 1. Composition of fish meal based (Z)-9-tricosene pellets

Ingredients (g)	Fish meal (FM) pellet	Fish meal + Pheromone (FMP) pellet	Fish meal + Pheromone + antioxidant (FMP) pellet
Fish meal	95.00	94.88	94.66
Molasses	5.00	5.00	5.00
(Z)-9-tricosene	-	0.22	0.22
Butylated hydroxy toluene (BHT)	-	-	0.22
Total Volume	100.00	100.00	100.00

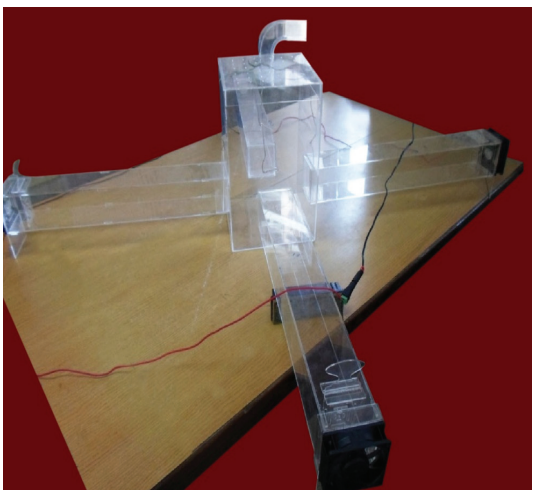

Fig. 1. Chemotactometer for testing pellet bait preference in house flies

Fig. 2. Glue traps used for testing pellet bait preference in house flies

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