Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 1071-1074

Received: 15-05-2020
Accepted: 10-08-2020

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Evaluation of (Z)-9-tricosene pheromone and food bait for house flies, *Musca domestica* L. (Diptera: Muscidae) attraction using Domo trap

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Abstract
Comparison of female sex pheromone, (Z)-9-tricosene and food bait tested in deep pit poultry unit in Bangalore, India, using blue coloured Barrix Domo trap. Targets treated with food baits of 30 g and 25g of 300 mg (Z)-9-tricosene gel formulation caught significantly greater number of both male and female than with (Z) -9- tricosene as positive control. Simple sugar, starch and protein baited targets were less attractive than control.

Keywords: pheromone, food bait, house fly, Z-9- tricosene

Introduction
Musca domestica, is a serious domestic, medical and veterinary pest that causes irritation, spoils food and is a vector for many pathogenic organisms. Adults occur on virtually all substrates surrounding animals including feed, faeces, vegetation, walls, and ceiling of buildings. The economic effects of house flies on livestock and poultry industries are substantial. Estimates indicate that stable fly and horn fly alone cause annual losses of 1.3 billion in reduced yields and increased production costs for beef and dairy industries [1]. High density of flies can cause stress to poultry workers, hens and affects the economic value of their products [2, 3]. Spraying of insecticides like DDVP and pyrethroids have been a successful fly control strategy worldwide [4]. Repetitive and indiscriminate use of these neurotoxic insecticides has led to resistance worldwide [5, 6, 7, 8, 9, 10].
Pheromone technology is an ecologically sustainable way of maintaining the fly populations below maximally acceptable limits.
There are several advantages compared to insecticide sprays as there is no exposure to humans and there are lesser chances of resistance development [11, 12]. (Z)-9-Tricosene can significantly enhance the catch rates of *M. domestica* in a wide range of trap types over a 24 h period [13, 14, 15, 16]. However, there are problems associated with the use of (Z)-9-tricosene as an attractant for *M. domestica*. One difficulty is that targets baited with (Z)-9-tricosene attract significantly more males than females [12]. Lure and kill systems that preferentially attract males will probably be less effective at supressing pest populations than strategies designed to attract both sexes or predominantly females [17, 18]. Therefore, the identification of semio-chemicals that attract females are essential in the design of fly control strategies based on odour baited targets. Volatile kairomones emitted from food or host sources may constitute more potent sources of attraction [18, 19]. The present investigation was carried out to evaluate the efficacy of pheromone and food baits for house fly attraction keeping in view the resistance development in house flies and to attract both the sexes of flies.

Materials and Methods
Trap Components
The target engaged in all the trials were the Barrix blue coloured Domo trap. The trap consisted of (1) base bowl, 200 ml capacity with four holes (17 *30 mm) for insect entry and pheromone disperse. (2) Collar, flat plate having projections at both sides and a circular hole at the centre (120mm). (3) Inverted cone with a height of 120 mm having two outer rims for gel application and (4) an outer transparent cylindrical enclosure with a height of 190 mm.

Experimental site

The field trials were conducted in a deep pit caged layer poultry unit in Bangalore (13°11'19.1724"N, 77°47'17.2176"E) India during 2017. The unit was composed of six identical houses situated opposite each other at 100m. Each house had a two-storey structure, the birds being housed on the upper floor and the manure contained in the pit below. It was 100m long and 30m wide. Both houses occupied 90,000 laying hens. There was a substantial opening to the manure pit below at both ends of the row of cages. These openings were the primary site of access to the upper storey for adult *M. domestica* emerging in the manure pit below. No other house fly control methods were encouraged during the trials in the above target experiment sites.

Pheromone formulation

The pheromone used in the trials was the synthetic female house fly sex pheromone, (Z)-9-tricosene synthesized in-house. The formulation consisted of technical 88% (Z)-9-tricosene, 6% (E)-9-tricosene and 6% impurities) incorporated in a crossed-linked polymer gel matrix at 25% w/w.

Armstrong void polymer (AVP) gel matrix formulation

AVP gel matrix includes microspheres zeolites (4 A^0) 0.99, gelling agent/polymer (Carbopol) 0.12%, moisture retaining agent 4.99%, antimicrobial agent 0.06%, UV blockers 0.07%, preservatives 0.06%, surfactant 0.5%, antioxidizing agent 0.99%, aqua 82.18% and (Z)-9-tricosene 1.15% used for the study.

Bait formulation

The target bait mixture was prepared by mixing flavouring ingredients which included simple sugars (33.3%), starch (33.3%) and protein hydrolysate (16.7%) with fermenting agent – yeast granules (3.33%) and anti-moisturizing agent (13.33%).

Experimental procedure

All the trials were conducted during the monsoon of 2017. Six replicates of each treatment were prepared and 48 targets were placed at the end of the cage rows, eight at each end of the house as random numbers. Blue coloured Domo traps were placed in the poultry shed in the ground level at 30-40 feet apart. The treatments were as follows:

Experiment 1

The first objective was to study the effects of the sugar, starch and protein-based food baits along with fermenting agent on the attraction of the flies. The traps were prepared by mixing 30g of each food bait separately with 150 ml of water and added to the base bowl.

Experiment 2

The second objective was to study the impact of incorporation of the house fly pheromone, Z-9 tricosene (300 mg) in gel formulation with the food baits (sugar, starch and protein) along with fermenting agent on the attraction of the flies. The traps were prepared by mixing 25g of pheromone gel formulation and 30g of each food bait separately. The pheromone gel formulation was added to the outer rim of the inverted cone and the food bait mixed with 150 ml of water to the base bowl.

Experiment 3

The last objective was to study the impact of the combination of Z-9 tricosene (300mg) in gel formulation and all the food baits (sugar, starch and protein) along with fermenting agent on the attraction of the flies. The traps were prepared by using 25g of pheromone formulation applied to the outer rim of the inverted cone and 30g of all the food baits mixed with 150 ml of water to the base bowl.

An effective concentration of 300 mg of Z-9 tricosene was selected based on the EAG studies and was used as positive control. The samples were collected every day by inverting the collecting transparent cone and emptying the contents into a field sample bag. *M. domestica* in the samples were identified, counted and the mean daily catches for each treatment were calculated. All the houseflies killed on a subset of sample dates within each trial were sexed by examining the external genitalia under a binocular microscope.

Statistical Analysis

The data of the total catches, male and female, of *M. domestica* obtained over the entire sample period of each trial were subjected to square root transformation and two-way analysis of variance (ANOVA). The mean values of the trials were compared using DMRT to determine if the attractant effect of the food odours or (Z)-9-tricosene varied significantly. Repeated measures ANOVA designs are appropriate when data has been collected from the same replicate on successive dates without rerandomization [20].

Results and Discussion

Experiment 1

The results on the mean (Table 1) of total catches over a week indicated that catches of *M. domestica* in the diverse traps varied significantly according to the treatments. The results showed that the protein bait with fermenting agent attracted slightly more number of *M. domestica* than starch and sugar baits. Protein and starch baits attracted more females than the sugar with fermenting agent (Table 1). However, there was no significant variation observed in male fly catches.

Experiment 2

The total catches with (Z)-9-tricosene, different food baits with fermenting agent combination revealed that there is no significant difference in the attraction of *M. domestica*. However, (Z)-9-tricosene with different food baits (protein, sugar and starch) and fermenting agent attracted more *M. domestica*, both males and females, than solitary treatments of these food baits and the pheromone as control (Table 1).

Experiment 3

The total catches of *M. domestica* were significantly enhanced by the (Z)-9-tricosene 300mg pheromone formulation with all the bait formulations. It also significantly increased the attraction of both males and females (Table 1).

The 300mg pheromone formulation as control increased the catch rates of *M. domestica* compared to the solitary treatment of different food baits (protein, sugar and starch) with fermenting agent. However, the effect of controls was less pronounced compared with all the other pheromone -bait combination treatments (Table 1, Fig.1 & 2).

Table 1: Total catches, female, and male of *Musca domestica* trapped in Barrix domo trap with pheromone and diverse food baits

Treatments	Total catches	Male	Female
Sugar and Fermenting agent	275.33^{g} (16.61)	102.50^{f} (10.14}	172.83^{d} (13.16)
Starch and Fermenting agent	389.17^{f} (19.74)	209.67^{e} (14.49)	179.50^{d} (13.41)
Protein hydrolysate and Fermenting agent	480.17^{e} (21.92)	286.00^{d} (16.92)	194.17^{d} (13.95)
Z-9 Tricosene, sugar and Fermenting agent	1051.83^{c} (32.38)	510.50^{c} (22.59)	541.33^{c} (23.20)
Z-9 Tricosene, starch and Fermenting agent	1484.00^{b} (38.51)	820.17^{b} (28.63)	663.83^{b} (25.73)
Z-9 Tricosene, protein hydrolysate and Fermenting agent	1531.50^{b} (39.10)	882.83^{b} (29.64)	648.67^{b} (25.44)
Z-9 Tricosene, sugar, starch, protein hydrolysate and Fermenting agent	6242.83^{a} (79.02)	4152.50^{a} (64.44)	2091.67^{a} (45.74)
Z-9 Tricosene control	890.83^{d} (29.86)	275.00^{d} (16.55)	615.83^{b} (24.81)
SEm	0.46	0.44	0.50
CD (0.05)	1.38	1.3	1.4

*Mean value of six replications. (Figures followed by same letters in the column do not differ significantly DMRT (P=0.05)
*Values in parentheses are square root transformed values

Fig 1: The Domo trap

Fig 2: A closer view of the catch

The catch rates of *M. domestica* with the (Z)-9-tricosene pheromone formulation, food baits formulation (sugar, starch and protein) and fermenting agent increased compared to the control and other treatments (Table1), indicating an increase in the attractive qualities of these baits over time. This conclusion was apparently elicited by the release of volatile products of protein putrefaction and carbohydrate fermentation of the food baits. Also, active cells in the yeast bait would have initiated fermentation of the sucrose added to the bait. The products of putrefaction and fermentation include many chemicals known to elicit the attraction of *M. domestica* [21, 22, 23, 24, 25, 26].

The results indicated that protein bait with yeast attracted more females. Females require protein for the complete maturation of their ovaries. Hence, it seems innate that the odours released from proteinaceous material may attract virgin females in considerable numbers [27]. (Z)-9-tricosene induced an increased attraction of *M. domestica* throughout the duration of the trial (Table1), as observed in previous trials [12]. This increase was primarily mediated by the response of the male *M. domestica*, though the numbers of females attracted to the (Z)-9-tricosene-impregnated targets were also significantly higher than food baits with yeast targets (Table 1).

Laboratory studies have demonstrated that (Z)-9-tricosene is highly attractive to the male *M. domestica*, but does not elicit female attraction [28, 29]. The increase in female attraction observed in this experiment corresponds with the findings of previous field trials [12, 13, 14, 15]. The targets treated with an Alfacron-sugar mixture and baited with 2.5 g of 40% (Z)-9-tricosene beads caught significantly greater numbers of both male and female *M. domestica* than the control targets [12].

Conclusion

The present study demonstrated that (Z)-9-tricosene pheromone gel formulation with all the different food baits attracted more *M. domestica* and this combination can be used as an effective strategy for housefly management.

Acknowledgement

The author acknowledges and appreciates the entire team of Barrix Agro Sciences Pvt. Ltd. Bangalore for their valuable inputs and support.

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