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Evaluation of baited eco-friendly delta traps for control of houseflies *(Musca domestica)* in the poultry farms

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Abstract

The present study evaluated delta traps baited with oviposition attractant mixture (OAM), (Z)-9-Tricosene and a combination of both (OAM+(Z)-9-Tricosene) in the control of houseflies in the poultry farm. The OAM was prepared with Ethyl palmitate, Ethyl linoleate, Methyl linoleate and Linoleic acid in 10:24:6:0.2 proportions. Traps were evaluated in two trials; in trial-I, OAM was prepared with liquid paraffin as solvent and in trial-II with acetone as solvent. In trial-I, the highest number of flies were trapped in OAM+(Z)-9-Tricosene baited traps followed by (Z)-9-Tricosene baited traps, OAM baited traps and control traps. In trial-II highest number of flies were trapped in OAM+(Z)-9-Tricosene baited traps, followed by OAM baited traps, (Z)-9-Tricosene baited traps and control traps. There was a significant difference ($p<0.01$) in the number of flies trapped with different baits. A clear variation in sex of flies trapped was observed with highest male to female sex ratio noticed in (Z)-9-Tricosene baited traps in both trial-I (3.44: 1) and trial-II (3.12:1) and lowest male to female ratio in OAM baited traps in both trial-I (1.69:1) and trial-II (1.39:1). Fly densities evaluated before and after the trial using spot cards revealed 12.4 and 6.8% reduction in the fly spots for trial-I and trial-II, respectively. Based on the observations, it can be concluded that baited delta traps can be effectively employed in integrated fly management strategies to control houseflies. OAM can be used as an efficient bait to trap female flies and can be combined with other baits like pheromone (Z)-9-Tricosene for increased effectiveness.

Keywords: *Delta traps, oviposition attractant mixture, (Z)-9-tricosene, housefly*

Housefly, *Musca domestica,* is predominant all over the world (Stensmyr, 2013), especially in poultry farms, livestock farms, household kitchen and slaughter houses (Ranjbar *et al*., 2016). They cause severe annoyance to animals, poultry and humans. Houseflies are implicated in the transmission of various diseases. Further, they also transmit the eggs of various helminths, cysts and trophozoites of protozoa (Adenusi and Adewoga, 2013). They act as an intermediate host for *Habronema muscae, Draschia megastoma, Thelazia rhodesii* and *Choanotaenia infundibulum* (Ponnudurai *et al*., 2003). Apart from these effects they also deposit faecal and vomit drops on places where they land which are commonly known as "flyspecks" especially on eggs, which increases the operational cost for cleaning the eggs and reduce the keeping quality of eggs (Douglas and Jesse, 2002).

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Most commonly used fly control methods are use of chemical insecticides like malathion, diazinon, dichlorvos, phosphamidon, cypermethrin, and carbosulfan as topical application in addition to baits and fumigants. However, houseflies rapidly develop resistance against these insecticides. Damage to the beneficial insects is also a challenge in insecticide-based control measures (Cluck, 1990; Oi, 1992; Kence and Kence, 1992). Hence alternate methods such as biological control, plant based essential oils and mechanical control are being followed (Kalsbeek *et al*., 2001; Koul *et al*., 2008; Fan *et al*., 2010; Tamilam *et al*., 2010, Pavela, 2013).

Mechanical control is commonly used in integrated fly management strategies. It involves the use of traps with different types of baits. Colour and baits used in the traps act as cues for attracting flies and play a major role in the effectiveness of traps (Diclaro *et al*., 2012; Khan *et al*., 2013). Housefly sex pheromone (Z)-9-Tricosene has been tested to attract flies in several laboratory bioassays and field studies, which proved its effectiveness (Lemke *et* al., 1990; Sundar *et al*., 2016; Sundar *et al*., 2018). The (Z)-9-Tricosene mostly attract male houseflies, so there is a need for bait that effectively attract female flies as well, which when used in traps will curtail the number of female flies thereby reducing number of active flies and subsequent generations. Several researchers have studied the oviposition ecology and behavior of houseflies (Jiang *et al*., 2002; Lam *et al*., 2007; Lam, 2010). Oviposition attractant mixture formulated with volatiles of fermented wheat bran tested in laboratory and field has shown promising results in attracting female flies (Tang *et al*., 2016). The present study was undertaken to formulate and test different baits and their combinations along with a durable, cost-effective and eco-friendly trap to effectively control houseflies in the field.

Material and methods

Preparation of bait

Oviposition attractant mixture (OAM) was prepared by mixing four chemicals *viz*., Ethyl palmitate (EP), Ethyl linoleate (EL), Methyl linoleate (ML) and Linoleic acid (LA) in 10:24:6:0.2 proportions, respectively (Tang *et al*., 2016). Liquid paraffin (light) and acetone were used as bases for preparation of OAM for trial-I and trial-II respectively. The final concentration of four chemicals was 1 µg/µL in Liquid paraffin/acetone.

Fifty mL of OAM was prepared by mixing 12.435 µL of EP, 29.844 µL of EL, 7.461 µL of ML and 0.248 µL of LA in 49.95 mL of Liquid paraffin/Acetone. Similarly, 6.8% of (Z)-9-Tricosene was prepared by mixing 680 µL of (Z)-9-Tricosene in 10 mL of acetone.

Bait dispensers

Vapour patches (VP) were obtained from Crawford Wisdom, Delhi for impregnation and sustained release of the baits. The VP were white coloured, circular devices with dimensions of 350 mm diameter, 150 gram/m^2 and made of non-woven fabric material (Fig 1). One side of the patch was provided with a self-adhesive material while the other side had a mesh meant for impregnation of bait.

Spot cards

Spots cards were used to assess fly density in the poultry houses. White executive bond papers were cut into 7.62 × 12.7 cm (3 × 5 inch) dimensions and stuck onto 2 cm thick thermacol of the same dimensions. Ten spot cards were prepared and fixed on stands of the cages using double sided tape (Fig. 2). Spot cards were placed for 24h before and after the trial. The number and location of spot cards was kept constant.

Enumeration of fly spots

Spot cards were placed for 24 hrs before and after trial and number of spots on each spot card were counted manually and recorded.

Delta traps

Delta traps were fabricated using red coloured fluted polypropylene sheet with slight modification of USDA standards. The delta traps were 28 cm in length, 15 cm width and 20 cm height. An insert with yellow sticky

Table 1. Experimental design

Groups	Trial	Baits	No. of traps
Control	I	500 µL of liquid paraffin	5
	II	500 µL of acetone	
OAM	I	500 µL of OAM (1µg in 1µL of liquid paraffin)	5
	II	500 µL of OAM (1µg in 1µL of acetone)	
(Z)-9-Tricosene	I&II	100 µL of 6.8 % (Z)-9-Tricosene in acetone	5
Combination (OAM + (Z)-9-Tricosene)	I	500 L of OAM (1µg/µL of liquid paraffin) + 100 µL of 6.8 % (Z)-9-Tricosene	5
	II	500 L of OAM (1µg/µL of acetone) + 100 µL of 6.8 % (Z)-9-Tricosene	
Total number of traps			20

Fig. 1. Vapour patches

Fig. 2. Deployed spot cards

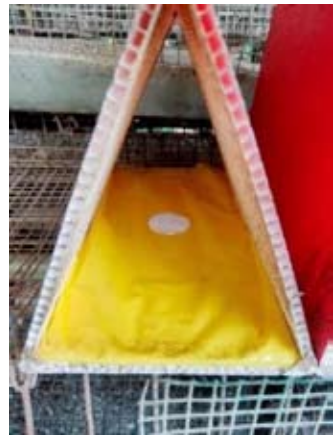

Fig. 3. Assembled delta traps

insect trapping glue sheet was placed inside delta trap. The insert dimensions were 27.5 cm length and 14 cm width (Fig. 3.).

Experimental site

Field assessment of baited delta traps was performed in the Japanese quail house at the Poultry Research Station, Madhavaram, Chennai. The dimensions of the shed were 27 meters in length, 10.4 meters in width, 4.88 meters in height at the centre and with 2.3 meters eaves height. The roof was fully monitored with a concrete floor. Birds were caged in two tier and three tier systems.

Trapped flies were collected along with a sticky sheet once in every three days and new sticky sheet was placed inside the trap. Six replications were carried out in a trial. The collected flies were counted and the ratio of male and female flies was determined by randomly examining the 10% of the flies trapped in each trap.

Statistical analysis

Results of the efficacy of baits were analysed using the Chi-square test in Graph pad Prism version 9 software and the variation in the male and female flies trapped was analysed using ANOVA and the means were compared using Duncan's multiple comparison test in SPSS software version 20. The spot card counts before and after the trial were compared with paired T-test.

Results and discussion

Trial-I

In trial-I, traps with OAM+(Z)-9-Tricosene lured the highest number of flies (6,234 flies, 32.90%), followed by traps with (Z)-9-Tricosene alone (4,965 flies, 26.20%), OAM alone (4,626 flies, 24.41%) and control traps (3,122 flies, 16.47%). The difference between the efficacy of baits in luring flies was noticed to be highly significant ($p<0.01$) with χ^2 (chi-square) value =484.91. The average number of flies trapped per trap per day (FTD) was highest in traps for combination of baits (69.26 flies), followed by (Z)-9-Tricosene baited traps (55.16 flies), OAM baited traps (51.4 flies) and control traps (34.6 flies) (Table 2).

The highest number of flies trapped in OAM+(Z)-9-Tricosene baited traps could be attributed to the additive effect of the baits. (Z)-9-Tricosene is a sex pheromone that mostly attract male flies and OAM attracts females. In addition, (Z)-9-Tricosene also acts as an oviposition

Table 2. Number of flies lured towards delta traps with different types of baits in trial I

Replication	Control		OAM		(Z)-9-Tricosene		Combination		Total
	No. of flies	% flies	No. of flies	% flies	No. of flies	% flies	No. of flies	% flies	
1	341	14.71	659	28.42	538	23.20	780	33.64	**2318**
2	549	20.74	489	18.47	605	22.85	1004	37.92	**2647**
3	945	20.26	902	19.33	1333	28.58	1484	31.81	**4664**
4	991	14.65	2070	30.60	1702	25.16	2001	29.58	**6764**
5	172	12.93	282	21.20	456	34.28	420	31.57	**1330**
6	124	10.13	224	18.30	331	27.04	545	44.52	**1224**
Total	3122	16.47	4626	24.41	4965	26.20	6234	32.90	18947
FTD	34.6		51.4		55.16		69.26		

χ^2 value =484.91** (between the baits) ** Significant ($p < 0.01$)

attractant for houseflies (Jiang *et al.,* 2002)**.** This additive effect by mixing two or more baits was observed in several studies, like Kannan *et al.* (2020), who observed trapping of more flies in traps baited with a combination of food bait and (Z)-9-Tricosene, Sundar *et al.* (2022) found that mixing of fish meal with (Z)-9-Tricosene was more effective than individual baits. Traps baited only with (Z)-9-Tricosene lured second highest number of flies (4965), which was more than OAM baited (4626) and control traps (3122). Studies with traps containing single bait proved that (Z)-9-Tricosene baited traps attracted a greater number of flies (Carlson *et al.,* 1971; Hanley *et al.,* 2004; Kannan *et al.*, 2020). In the present study, significantly more flies were trapped in OAM baited traps (4626) than in control (3122), indicating the luring efficacy of OAM baited delta traps.

Trial-II

In trial-II, traps baited with OAM+(Z)-9-Tricosene lured 5,462 flies, which was highest among all baits, followed by OAM baited traps with 5,053 flies, (Z)-9-Tricosene baited traps with 4731 flies, and control traps with 3690 flies. Statistically, the difference in number of flies trapped with different baits was highly significant ($p<0.01$) with $\chi 2$ value =1383.10. The FTD for traps baited with control, OAM, (Z)-9-Tricosene and a combination of baits was 41, 56.14, 52.56 and 60.68, respectively (**Table 3**).

Acetone has been widely used as a solvent for preparing solutions of non-polar nature. Housefly pheromones such as (Z)-9-Tricosene are commonly prepared with acetone (Hanley *et al*., 2004; Sundar *et al*., 2014). However, no literature is available on the usage of acetone for the preparation of OAM. In this study, OAM was prepared with acetone and its luring efficacy was tested in poultry unit using delta traps.

Significant variation was observed upon comparing the similar types of baits of trial I and II (Tables 2 and 3). $\chi 2$ values of control, OAM, (Z)-9-Tricosene and combination traps for the number of flies trapped between trial I and II were 58.15**, 25.64**, 7.41** and 73.07**, respectively, indicating a highly significant difference. The percentage of fly trapping increased for control and OAM baited traps in trial-II compared with trial-I.

The findings revealed that OAM prepared with acetone as a solvent had more efficacy compared to that prepared with liquid paraffin. In trial-II, similar to trail-I more number of flies were trapped in a combination of baits (5462). In contrast to trial-I, a greater number of flies were trapped in OAM baited traps (5053) in trial-II compared to (Z)-9-Tricosene baited traps (4731). The difference was also noted between OAM baited traps of trial-I and II. This could be due to the use of acetone as a solvent in trial-II, which could have facilitated better dissemination of OAM due to its highly volatile nature. However, the possibility of the presence of female biased fly population cannot be ignored (Sundar *et al.* 2022).

Variation in the sex of trapped flies

The total number of male and female flies trapped varied with the type of bait used. In both trial I and II, highest male to female ratio was observed in traps baited with (Z)-9-Tricosene followed by control traps, traps with combination of baits and OAM baited traps (Table 4). The trapping of house flies in different baited traps could be influenced by the population density and sex ratio of flies available in the study area.

The mean (±SE) values for per cent of male and female flies trapped in different baited traps in both trials is given in Table 4. The means when compared between the groups using Duncan's multiple comparison test revealed a highly significant difference ($p<0.01$) in the mean per cent of male and female flies trapped in both the trials between control and OAM; (Z)-9-Tricosene and OAM; (Z)-9-Tricosene and Combination of baits.

In the present study, a great variation was noticed in the number of male and female flies trapped with different baits. Overall, a greater number of male flies were trapped in all traps compared to females. This could be due to higher locomotor activity of male flies compared to females

Table 3. Number of flies lured towards delta traps with different types of baits in trial II

Replication	Control		OAM		(Z)-9-Tricosene		Combination		Total
	No. of flies	% flies	No. of flies	% flies	No. of flies	% flies	No. of flies	% flies	
1	209	15.92	370	28.20	337	25.68	396	30.18	1312
2	758	20.00	1024	27.02	880	23.22	1127	29.74	3789
3	974	21.04	1325	28.62	1048	22.63	1282	27.69	4629
4	1000	20.61	1247	25.70	1351	27.84	1254	25.84	4852
5	615	19.49	842	26.68	788	24.97	910	28.84	3155
6	134	11.17	245	20.43	327	27.27	493	41.11	1199
Total	3690	19.48	5053	26.68	4731	24.98	5462	28.84	18936
FTD	41		56.14		52.56		60.68		

χ^2 value =1383.10**(between the baits) ** Significant ($p < 0.01$)

Table 4. Means of per cent sex variation in flies trapped in different baited traps in trial-I and II

Group	Trial-I			Trial-II		
	Males (Mean ± SE)	Females (Mean ± SE)	Sex ratio	Males (Mean ± SE)	Females (Mean ± SE)	Sex ratio
Control	69.58^{ab} ± 3.79	30.41^{bc}± 3.79	2.5:1	69.07^{ab}± 3.19	30.92^{bc}± 3.19	2.12:1
OAM	58.85^{c}± 3.21	41.14^{a}± 3.21	1.69:1	59.82^{c}± 1.72	40.16^{a}± 1.73	1.39:1
(Z)-9-Tricosene	75.70^{a}± 2.18	24.28^{c}± 2.18	3.44:1	75.54^{a}± 4.19	24.45^{c}± 4.19	3.12:1
Combination	63.43^{bc}± 3.10	36.57^{ab}± 3.10	1.98:1	61.96^{bc}± 2.05	38.03^{ab}± 2.05	1.61:1
p value	0.006	0.006		0.005	0.005	

Means with different superscripts within the same column differ significantly from each other ($p < 0.01$)

(Ragland and Sohal, 1973; Buchan and Sohal, 1981; Bahrndorff *et al*. 2012). It could be also due to high male to female fly ratio in the study area during the study period. The male biased fly population can be complemented with the fact that even in control traps a greater number of male flies were trapped as against female flies.

The per cent of female flies trapped was more for OAM baited traps compared to any other trap (Table 4), indicating the female fly preference to OAM. Similar results were observed by Tang *et al.* (2016). The sex ratio in the (Z)-9-Tricosene +OAM baited traps was more than OAM baited traps. This might be due to the attraction of both males and females towards (Z)-9-Tricosene as it also acts as an aggregation pheromone (Sundar *et al.,* 2017) and attraction of female flies towards OAM.

A higher percentage of male flies were trapped in (Z)-9-Tricosene baited traps compared to any other traps and these observations are in agreement with Hanley *et al.* (2004) and Sundar *et al*. (2018). In contrast, several authors have reported trapping of more female flies in (Z)-9-Tricosene baited traps (Carlson and Beroza, 1973; Morgan *et al.,* 1974; Mitchell *et al.,* 1975; Sundar *et al*., 2022). These variations could be due to different sex ratio in the field and environmental temperatures, which affect the number and activity of flies thereby the number of male and female flies trapped (Buchan and Sohal, 1981).

Estimation of fly densities before and after trapping

Estimation of fly densities is essential for assessing the rate of infestation and evaluating the efficacy of the control measures employed. Spot cards are commonly used to assess the indoor densities of housefly populations (Beck and Turner, 1985; Ponnudurai and Harikrishnan, 2011; Gerry *et al.,* 2011; Machtinger and Burgess, 2020). Spot cards indirectly help to estimate the activity status of houseflies in animal houses.

In the present study of fly spots recorded in spot cards which were placed before and after trial-I was 4,386 and 3,839, respectively and in trial II it was 4,400 and 4,119, respectively. The mean (±SE) values for trial-I (54.7±60.91) and trial-II (28.1±27.14) were not statistically significant (P>0.05).

In both the trials lower number of fly spots were recorded after the trial (3839, 4119) compared to before trial (4386, 4400). There was a 12.4% reduction in fly population in trial-I and 6.8% reduction in trial-II. Similar observations were recorded by Gerry *et al.* (2011), Sundar (2017) and Sathiyamoorthy *et al.* (2018). The average fly spots in spot cards recorded before and after trial-I was 438 spots/card/day and 383/card/day, respectively and for trial-II was 440 spots/card/day and 411/card/day, respectively. According to Stafford (2008), presence of 50 spots/card/week indicates the need for control measures and 100 spots/card/week indicates very high infestation. Based on these indications, the fly density in the present study was very high. Though the spot card counts before and after the trial indicated a decrease in fly population, fly densities were still very high after the end of trial.

In the present study, though baited delta traps effectively trapped flies to bring down the fly population, fly densities were still high. All traps were found saturated with flies. Evaluation of more delta traps or use of other control measures along with traps may be required to effectively trap more flies, thereby reducing the fly population to permissible levels. Integration of traps with other control measures are needed for effective housefly control (Snell, 2002; Chapman *et al*., 1998a, 1998b).

Conclusion

Based on the present findings, the study concluded that, delta traps used were found to be effective, economical, durable and eco-friendly. The bait combination (OAM+(Z)-9-Tricosene) was also found effective in luring houseflies towards delta traps. The delta traps and baits can be employed in the integrated fly management for effective control of flies in the poultry farm.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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