



## Research Article

# Recent research in pheromone trapping towards bio-intensive management of major insect pests in agro-ecosystems of India

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**ABSTRACT:** With the discovery of insect pheromones in intra-species communication in insects several decades back, the R&D on deploying synthetic insect pheromones as mimics of these olfactory signals is being increasingly explored for eco-friendly management of insect pests in major Agri-horticultural and forestry ecosystems. The focus of this paper is on the twin dimensions of pheromone deployment, namely monitoring the pest/vector populations and their utility in mass trapping for population suppression. The relevant pheromone trapping systems R&D scenario in India is illustrated with outcomes mainly from our on-going collaborative research. In particular, it has opened up the scope to identify more efficient pheromone trap design by clarifying that funnel/sleeve trap seems adequate to trap noctuid moths, while a modified Delta trap (Delta-Plus) is clearly superior for at least three Crambid moths, with focus on trap attributes to improve male moth arrivals and/or minimize their escape tendency. The scope for integration of pheromone technology with biological control towards bio-intensive IPM is indicated. Further, the policy support environment and future requirements are also covered in this paper.

**KEY WORDS:** Dispenser types, integration, IPM, mass trapping, pheromone trapping, R&D, trap design

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## INTRODUCTION

The use of para-pheromones for tephritid fruit flies is gaining importance globally with a focus on widening the application for monitoring for quarantine and local timing of control interventions, including blending and alternative trap-dispenser options (Shelly *et al.*, 2004; Varagas *et al.*, 2009). The utilization of lepidopteran pheromones is also making strides, moving from the phase of identification, characterization and synthesis to trap-lure combinations, besides alternative dispensing options and also the evolutionary aspect of intra-species diversity in response to female pheromones (Groot *et al.*, 2018).

### At national level

In India, the major public sector research impetus has been on identification, characterization and synthesis of moth pheromones, especially by Council for Scientific and Industrial Research (CSIR), research institutes under Indian Council of Agricultural Research (ICAR), besides academic-research institutions like Tamil Nadu Agricultural University (TNAU) etc. Relevant research on the efficient deployment of the synthetic pheromones in target crop ecosystems has made significant progress in cereals, pulses, oil seeds, vegetables, fruit crops, and plantation crops. For para-pheromone-based trapping of tephritid fruit flies, the contribution of the ICAR-DFID network program implemented about a decade ago was substantial, covering the

aspects like trap design attributes and alternative dispenser systems involving methyl eugenol for the species complex led by *Bactrocera dorsalis* in mango and guava ecosystems, as well as cue-lure for trapping the species complex led by *B. cucurbitae* among the cucurbit vegetable crops (Stonehouse *et al.*, 2005). For lepidopteran pheromones, the use of sex pheromone traps has been studied widely in last four decades, with early focus more on identification, characterization and synthesis of the components of the female sex pheromone and about phenotypic plasticity among geographical populations in response to blend ratios as in *Helicoverpa armigera* and *Leucinodes orbonalis*, while recent emphasis has been more on alternative trapping and dispensing system options, especially on trap design and lure dispensers (Nandagopal *et al.*, 2008).

The research and development (R&D) wing of Sun Agro Biotech Research Centre, Chennai in collaboration, especially with TNAU, CSIR and ICAR institutions has focussed on identifying alternative trap designs and dispenser systems. For methyl eugenol-based fruit fly trapping, the use of cylindrical white jar traps in combination with vial-wick dispenser has been found promising, while for cucurbit vegetables, there is scope for alternative trap types, pheromone blend and lure dispenser deployment (Amsa *et al.*, 2014; 2015; Divya *et al.*, 2011; Sithanantham, 2011; Suganthi *et al.*, 2014). Further, it has been clarified that while the sleeve (funnel) trap is still efficient for trapping noctuid moths like

*Helicoverpa*, it is found to be clearly inefficient for the crambid moth- *Scirpophaga incertulas* in rice compared to the improved delta traps, with additional access vents (Delta-Plus), which are found to be also superior to both sleeve traps and water basin traps for *Leucinodes orbonalis* in brinjal (Prabakaran *et al.*, 2017) and *Chilo infuscatellus* in sugarcane (Prabakaran *et al.*, 2018).

Multi-location mass trapping impact optimization studies are being pursued for rice yellow stem borer with the improved delta (Delta-Plus) traps with TNAU collaboration. Similar efforts have been made under the Tamil Nadu Innovative Initiatives (TANII) program for large area popularisation of trapping of mango fruit flies with the cylindrical jar traps and vial-wick dispenser combination in Krishnagiri-Dharmapuri districts recently covering about two thousand mango farmers over three years culminating in overwhelming economic and ecological benefits (Ramaraju *et al.*, 2018). The TNAU-led multi-location network monitoring of fruit flies, with SABRC collaboration, has evolved as a viable model for assembling baseline data on seasonal and locational diversity of the species complexes responding to methyl eugenol and cue-lure, to guide area-based strategies (Elayabarathy *et al.*, 2018).

Hence attempts were made to identify more efficient pheromone trap design with focus on trap attributes to improve male moth arrivals and/or minimize their escape tendency.

## MATERIALS AND METHODS

This communication illustrates our recent and on-going collaborative R&D on the following major thrusts:

### Selecting improved trap- lure combination for fruit flies

This study was carried out during Dec. 2015-Feb. 2016 in an organic cucurbit farm near Coimbatore, Tamil Nadu, comparing the following eight treatments.

- T1. Jar trap plus vial with wick
- T2. Jar trap plus large size (4x4x1cm) disc
- T3. Jar trap plus medium size (4x2x1cm) disc
- T4. Jar trap plus small size (4x1x1cm) disc
- T5. Delta trap plus large size (4x4x1cm) disc
- T6. Collapsible trap plus large size (4x4x1cm) disc
- T7. Jar trap plus vial with wick\*
- T8. Jar trap plus large size (4x4x1cm) ply wood disc\*

(\*Lure type (Cue lure) common for first six treatments, with special lure type in last two treatments).

This study involved comparing the treatments positioned at about 20 metres in between in four replications. The trap catches were recorded weekly for ten continuous weeks, with random interchange of their positions within each replication, for avoiding position effects.

### Evaluating alternative trap designs-dispenser options for moth borers

The field studies on pheromone trap types and lure loadings were taken up in a farmer field near Thiruvallur, Tamil Nadu during 2015-16 crop seasons.

The traps compared were four variants of Delta trap, besides water basin trap, circular plate trap and funnel (sleeve) trap. They were kept at inter distance of 10 meters each in three replications and the synthetic sex pheromone was dispensed in rubber septa. The trap catches were recorded weekly for four weeks and the trap positions within the replication were inter-changed at random at each week.

### Study of insect orientation and distribution in pheromone trap sticky arena

The orientation of male moths of *Leucinodes orbonalis* in the sticky arena of delta trap was also studied in the above trial, by examining the sticky sheets collected in four weeks from different replications in the same trial. The moths' orientation in relation to the lure source was assorted into three categories, towards the lure, directly opposite to the lure and sideways away from lure.

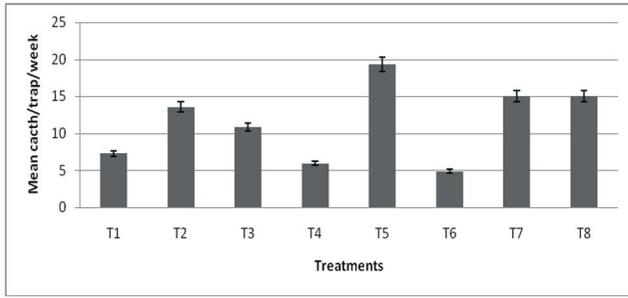
### Relative distribution of moths in different sections of catch arena

The same study was also extended to assess the relative catches of the male moths in different sections of the catch arena positioned along the length and width of the sticky liner sheets in the delta trap by examining pheromone trap gum sheets (liners) collected in the same trial. The moths caught in positions were tallied among four columns (parallel to the trap length) and six rows (parallel to trap width).

## RESULTS AND DISCUSSION

### Selecting improved trap -lure combination for fruit flies

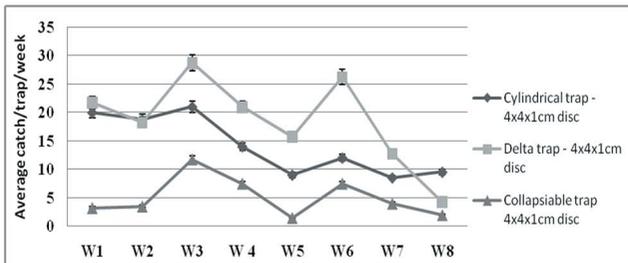
The results showed significant overall differences in catches among the eight treatments (trap type-lure dispensers combinations). The maximum mean catch ( $19.35 \pm 5.13$ ) was in the combination of improved delta trap with large disc (4x4x1cm) when Cue lure (CL) was deployed alone (Fig. 1). This was also significantly greater than the next two combinations for blended cue lure, being cylindrical trap with vial-wick dispenser ( $15.07 \pm 4.45$ ), which was also on par with same trap type and large disc (4x4x1cm) combination



**Fig 1. Comparison of traps design with lure dispensers**

(15 ± 5.94) when blended with ME (Fig. 1). The next ranking two combinations for unblended CL in cylindrical jar traps were large disc (4x4x1cm) (13.22 ± 4.94) followed in rank by medium disc (4 x 2 x 1cm) (10.82 ± 4.58). The other three combinations were rather poor in catches and hence did not appear promising, all for unblended CL, namely vial in jar trap (6.85 ± 4.21), small disc (4 x 1 x 1cm) in jar trap (5.95 ± 2.20) and large disc (4 x 4 x 1cm) kept in the collapsible trap (4.87 ± 3.00).

As combinations for the best dispenser, namely the large disc (4x4x1cm), the delta trap type trap (Delta-Plus) was found to be significantly superior to the alternative trap types in at least six out of the eight week period of observation (Fig. 2).



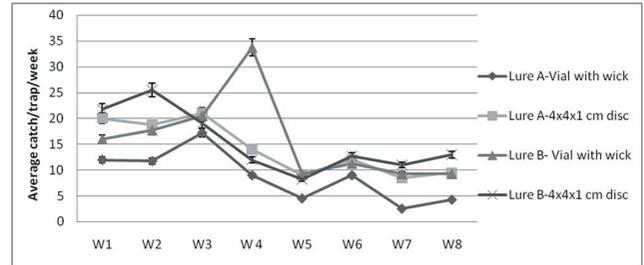
**Fig 2. Comparison of trap catch in jar trap between alternative lure dispensers (4x4x1cm disc)**

This impressive superiority of Delta trap as an alternative to the hitherto promising cylindrical jar trap is an important information. Further, this result is also in conformity to similar findings of superiority of Delta trap over the cylindrical jar type observed by Suganthy *et al.* (2014) when comparing them of course with the smaller disc (4 x 1 x 1cm). Of course, there is need and scope to ascertain whether such trend is also likely with alternative dispenser types for the individual species in the *B. cucurbitae* complex.

Further, the relative trap catches in the cylindrical trap for unblended cue lure (A), followed a descending order for the dispensers as 4 x 4 x 1 cm disc > 4 x 2 x 1cm disc > vial with wick > 4 x 1 x 1cm disc, suggesting that when CL alone was dispensed, the catch could be maximised by large disc

and not by vial with wick (Fig 3) or other disc sizes. This result is also in conformity with the results of Suganthy *et al.* (2014).

The consistent superiority of large disc over vial in the cylindrical jar types was clearly evident in unblended cue lure (lure A), rather than in blended Cue lure (Lure B) (Fig. 3).



**Fig 3. Comparison of trap combination with alternative lure dispensers for unblended (A) and blended (B) cue lure**

Our present results are also in agreement with earlier studies by Suganthy *et al.* (2014) which had indicated that the extent of difference in catch between blended and non-blended cue lure for overall catch of fruit flies was more for smaller disc rather than for larger disc. It is therefore evident that the consistency of superior trap-lure combinations needs to be further studied for blended Cue lure among alternative lure dispensers.

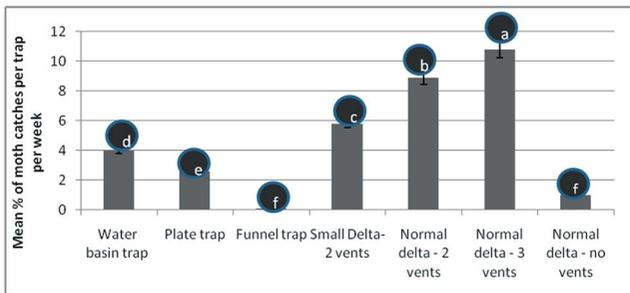
The present results have clarified that as trap type combination for the same lure dispenser and CL blending deployed, there was overall more catches indicating more attraction and/or retention of the *B. cucurbitae* males in the hitherto recommended cylindrical jar trap, compared to latter two trap types. The present results with cue lure for melon fly trapping also indicate that cylindrical jar traps in combination with large disc (4 x 4 x 1 cm) caught more fruit flies (7.0) compared to vial with wick (2.5), which is also in conformity with the findings of Divya *et al.* (2011) and Suganthy *et al.*, (2014), besides concurring also with the findings of Gajalakshmi *et al.* (2012) that this same combination was also superior for methyl eugenol (ME) attractant deployed to trap the *B. dorsalis* complex. The presently observed superiority of the combination of large disc (4x4x1cm) in cylindrical white jar trap (15.4) for melon fruit fly, which was followed in rank by combination with delta trap (11.3) and the collapsible trap (6.8), respectively, is also comparable to findings of Suganthy *et al.* (2014).

Interestingly, the benefit of blending CL with ME, although appearing to be greater with large disc dispensers (15.3) compared to vial dispensers (13.8), both were on par. Therefore, for blended CL (with ME), both these lure dispensers can be reckoned to be comparable in enabling

increased catches of *B. cucurbitae*. Our results are also in conformity with the report by Shelly *et al.*, (2004) on differential performance among lure dispensers in contrasting trap types for *B. cucurbitae* and *B. dorsalis* in Hawaii. Further studies on the adult behaviour in relation to trap design attributes and the nature of synergy effects of blending of Cue lure on related neuro-physiological responses are recommended so to provide further insight into the interactions of trap design and dispenser types in the context of cue lure being deployed alone versus as blend (with methyl eugenol) towards maximising the fruit fly trap catches in cucurbit ecosystem.

**Evaluating alternative trap designs-dispenser options for moth borers**

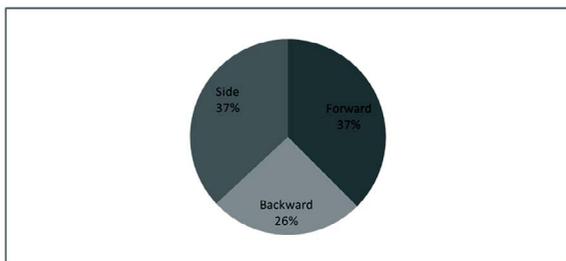
The overall trap catches differed significantly among the trap types. The maximum catches were in the normal size delta trap with three access vents, followed by the same size with two access vents, while small size delta trap with two access windows was next in rank and all these three types we distinctly superior to the normal delta without access vents (Fig. 4). The overall performance of remaining trap types compared in descending order of moth catches were: water basin trap > plate trap > delta trap without access vents > funnel trap.



**Fig 4. Relative catches of *Leucinodes orbonalis* in six trap types in brinjal field**

**Study of insect orientation and distribution in pheromone trap sticky arena**

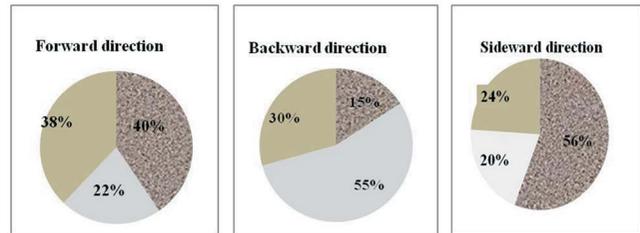
The overall proportion of moths caught with their heads towards the lure (forward) was only about 37 per cent of the total (Fig. 5), whereas another 37% were oriented sideways,



**Fig 5. Relative share of three moth orientation directions in sticky arena of delta trap**

besides about 26% oriented backwards (opposite to the lure).

The results have shown that only one thirds of the moths were found landing toward the lure (forward) whereas the remainder were oriented towards other two directions (backward, sideways), indicating predominant tendency to escape. The relative share of the three direction of orientation among traps with overall moth catches per arena being high, intermediate and low is also illustrated in (Fig. 6).



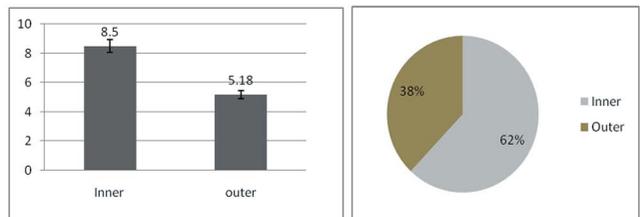
**Fig 6. Relative predominance of male moth orientation in three directions (High; Intermediate; Low)**

These results are also unique and provide a new dimension in our studies on male moth escape behaviour after arriving in the trap.

**Study of relative moth catch distribution in sections within the trap arena**

This study was to understand the pattern of landing of the moths in different positions within the sticky arena in pheromone trap. The results showed that the differences in numbers caught in four columns in length side and eight rows in width side differed significantly.

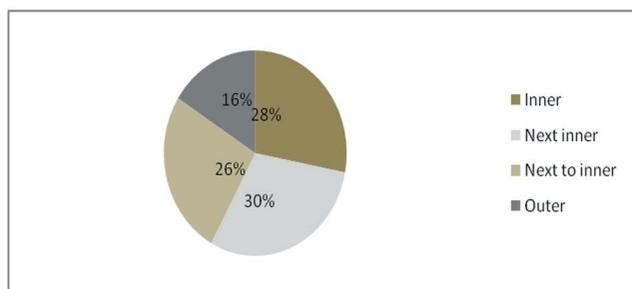
The mean numbers of moths caught in the inner two columns were 8.5, while those in the outer two columns were 5.2, respectively (Fig. 7). The relative proportion of males caught in the inner two columns was about 62%, compared to about 38% in the outer columns.



**Fig 7. Relative moth catches in two sections on the length side of trap liner (BS FB)**

In the width side of the sticky arena, the different sections showed varying frequency of moths trapped (Fig 8). The inner-most, next to inner, next to outer and outermost sections accounted for about 28, 30, 26 and 16 per cent of the moth catches. The relative catches for the two inner sections being about 58 per cent while the three inner sections

excluding the outermost section accounted for about 85 per cent catches (Fig 8).



**Fig 8. Relative share of trap catches in four sections on width side of trap liner**

These results can cater to selective readjustment in width, by excluding the outermost section, with still 85 percent catches being possibly secured with less sticky arena area.

The recent studies on pheromone trap design and dispenser loading being major factors in optimising the trap catches of moths have been illustrated herein and yielded adequate information towards holistic improvement in the trapping system impact potential. While the related ecological knowledge provides indication of the timing of emergence of female moths tends to be more spread out than males, besides the female dominant sex ratio observed in two local populations of *Leucinodes orbonalis* in Tamil Nadu (Preethi *et al.*, 2014a), complement the outcomes of field testing of four different trap types and lure dispensers towards improving the catches by selecting superior lure-design combination as reported by Preethi *et al.*, (2014b).

The major outcomes of our collaborative research have led to the following original contributions:

1. The demonstration of white cylindrical jar trap with vial-wick dispenser combination as superior to alternative options in tree fruit ecosystems (using methyl eugenol) and confirmation of potential for improved Delta trap with plywood disc dispenser in Cucurbit vegetable ecosystems.
2. Demonstration of improved Delta trap with additional access vents (Delta Plus) as clearly superior to funnel (sleeve trap) for rice yellow stem borer, besides also superior to water basin trap for brinjal shoot and fruit borer and sugarcane early shoot borer.
3. Clarification of male moth escape tendency (after arriving in the trap) among three moth borers and so clarifying the interaction of olfactory and visual responses in maximising the trap efficiency.

4. The existence of intra-species genetic specialisation in cucurbit fruit fly, expressed as preferential response to host plant semio-chemicals in females, while males are indifferent to same stimuli.

### Scope for integration with other strategies

#### Pheromone trap as monitoring tool

In case of native pests, based on trap catch fluctuations, there is potential for integrating with augmentative bio control in optimum timing and dose refinement, while as quarantine tool it can also help alerting the incursions from invasive pests.

#### As tool in local mass trapping

Besides the complementary impact of reducing the oviposition (egg numbers) in the target crop habitat, the synergy benefit from augmentative release of the egg parasitic-*Trichogramma* can be captured as in brinjal, rice and sugarcane ecosystems.

#### As component of push-pull strategy

There is good scope for availing para-pheromones as pull source for fruit flies and synthetic female pheromones for moth borers

These strategies may constitute strong planks for the green technology popularisation.

#### Vision for future R&D

There is great scope for strengthening public-private-partnerships in R&D to evolve more of win-win models. More of inter-disciplinary collaboration among chemists, behavioural entomologists, nanotechnologists, molecular biologists and field ecologists can significantly enhance the pheromone technology applications. The thrust areas may include:

- i) Pheromone blend characteristics monitoring in geographical populations and crop habitats as basis for fine tuning the commercial lure composition across major agro-ecologies of target crops.
- ii) Ensuring maximum trap catches per unit quantity of synthetic pheromone dispensed by suitable selection of trap attributes, based on male insect behaviour studies, involving olfaction and vision.
- iii) Exploring for alternative dispensing systems and formulations which maximise the trap catches without requiring higher air thresholds of the target pheromone.
- iv) Assessing the potential safety of pheromone trapping technology in terms of native beneficial biodiversity and

potential for induced adaptation of target populations to higher thresholds.

- v) Enhancing the utility of pheromone-based trapping systems as key components in market focus cultivation of crops, besides extending to storage and forestry insect pest management.

### Policy support environment

The presently available national level regulatory guidelines are positive and cater to the motivation for commercial investment. The present policy of minimal registration requirements when used in monitoring or mass trapping versus more rigorous safety data requirements applicable to mating disruption and other systems of deployment which deploy higher air concentration of pheromone appears to be well balanced. Nevertheless, there is scope to periodically fine tune and/or revise the descriptors and types of traps and dispensers, besides evolving appropriate methodologies and parameters relating to habitat safety, as related to registration-related information applicable to importing or local manufacturing/marketing of pheromone technology components. More financial incentives should be extended since this green technology contributes directly to native biodiversity conservation in agro-ecosystems, besides catering to enhanced safety of consumers and the human environment by reducing the exposure/residue risks associated with chemical insecticide use.

### Recommendations for R&D and policy support

Based on the vast potential for pheromone technology to evolve as a key component of sustainable insect pest management in agri-horticulture besides stored commodity and forestry protection in India, more of collaborative R&D across public-private institutions and closer involvement of the stakeholders should be encouraged by suitable funding and human resource development initiatives, including a national co-ordinated R&D network under ICAR/CSIR patronage to evolve more robust and wider array of the technology options.

Policy issues governing pheromone technology regulatory guidelines, especially the quality assurance and eco-safety risk assessment requirements to be kept more vibrant and more holistically fine-tuned by fortifying the relevant experts' panels with multi-dimensional expertise available across public-private sectors. The policy support environment should be further improved with incentives for potential entrepreneurs based on the socio-economic and

ecological-human health benefits accruing from this green technology.

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