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Dose Optimization in a Wind Tunnel to Determine the Effective Concentration preferred by Male Fall Armyworm Moths

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ABSTRACT: The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), an invasive pest, has posed a concern to farmers and Indian agriculture after being discovered in maize fields on the Indian subcontinent in May 2018. Olfactory signals are commonly used as essential stimuli or releasers for activating responses such as mating partner orientation, identification of suitable oviposition sites, and foraging. A practical monitoring strategy is essential since early detection of the pest in the field will allow farmers to choose appropriate pest management strategies. To do this, a sex pheromone blend was prepared that is anticipated to outperform the commercially available lure. To test this theory, dosage optimization tests were carried out to determine an optimal dose preferred by male Fall armyworm moths. Based on the findings, field trials for effective monitoring, mass trapping, and integrated pest management practices can be implemented.

Keywords: *Spodoptera frugiperda*, Sex pheromones, Pheromone blend, Pest Management, Olfactory chemoreception.

INTRODUCTION

Olfactory-induced responses in moths have been related to reproduction, foraging, and feeding environments. Odorants that influence moth behaviour are characterised as either species-specific pheromones or generic odours. While olfaction is the primary modality in reproductive behaviour, while approaching flowers, visual signals might take preference (Balkenius *et al*., 2006). Sex pheromones emitted by female moths are similar to those emitted by males, but their chemical makeup is different from that produced by males (Butenandt and Von, 1959; Kramer, 1975). Male moths employ distinct ways of detecting pheromone sources. Flying moths have a distinctive orientation toward pheromone sources, which is validated by optomotor anemotaxis (Kennedy and Marsh, 1974).

Behavioural characteristics differ by species and are also affected by pheromone (blend) concentration (Bau *et al*., 2002; Cardéand Hagaman, 1979; Kuenen and Baker, 1982; Mafra-Neto and Cardé, 1995; Willis and Arbas, 1991). Similarly, effective pheromone source detection is dependent on the moth's exposure to temporally variable pheromone concentrations (Baker *et al*., 1984; 1985; Kennedy *et al*., 1980). Thus, to increase the legitimacy of the research, it is vital to conduct dosage optimization studies when working with synthetic chemical compounds.

After rice and wheat, maize is India's third most common food crop. Maize is grown throughout the year in all states for various reasons, such as grain, fodder, green cobs, sweet corn, baby corn, and popcorn in peri-urban regions (Farmer's Portal, 2021). Since the discovery of the invasive insect fall armyworm in maize in May 2018, it has spread rapidly over India's maize-growing ecologies, owing to its high dispersion capacity and affinity for warmer climates (Suby *et al*., 2020). Hence, to monitor the fall armyworm in the field, an effective sex pheromone blend is required that may easily stimulate male response to offer necessary knowledge to the farmer about the current diversity present and will be able to adopt necessary management strategies accordingly.

In anticipation of this scenario, a sex pheromone blend was prepared that is expected to be more potent than commercially available lures. However, to perform research on this, an appropriate concentration of the blend must also be determined, which will aid in the commercialization of the product. To accomplish this, wind tunnel experiments were conducted with varying dosages of pheromone blends to advance an idea for the

production of an efficient sex pheromone trap for monitoring and mass trapping.

MATERIALS AND METHODS

Insect Rearing. Insect eggs were collected from the Fall armyworm laboratory, Department of Agricultural Entomology, TNAU, Coimbatore. They were reared on an artificial diet developed by TNAU. They were kept at a temperature of 26 ± 2 °C with a relative humidity of 55 ± 5% and a photoperiod of 16 L: 8 D hours. The larvae were transferred into individual ventilated vials (20 × 15 × 8) for pupation when they reached the third instar stage. They were subsequently sorted out based on their reproductive organs under a stereomicroscope after adult emergence (Rwomushana, 2019). The adults were reared in transparent rearing cages (50 × 50 × 50), and a honey-sugar syrup solution was supplied as an adult diet using a cotton pad. Moths were reared in the lab for three generations before experimentation. All experiments were performed on three-day-old virgin male moths. Adult moths were used only once and were not exposed to any synthetic odour sources before the test.

Synthetic Chemical Compounds. The sex pheromones were selected based on the findings of earlier research by (Gargi, 2021), which primarily focused on the EAG analysis of the response of *Spodoptera frugiperda* moths to the sex pheromone blend. Compounds were acquired from Sigma-Aldrich, and experiments were conducted using a sex pheromone blend, which included ((Z)-9-tetradecenyl acetate (Z9-14: OAc), (Z)-7-dodecenyl acetate (Z7-12: OAc), and (Z)-9-dodecenyl acetate (Z9-12: OAc) in a ratio of 85:10:05), and a commercial *S. fugiperda* lure was also purchased from Pest Control India.

Preparation of chemical dosage. All sex pheromone standards purchased were diluted to different concentrations to determine the effective concentration preferred by male *S. frugiperda* moths. All of the compounds were diluted using HPLC-grade hexane as the diluent. All dilutions of various sex pheromone standards were carried out using the following equation:

$$\text{Concentration of stock soloution } (\mu g/ml) = \frac{\text{Weight of standard taken (mg)} \times \text{Purity\%} \times 1000}{\text{Volume to be made (ml)} \times 100}$$

Experimental Procedure. A wind tunnel (200 cm length × 75 height × 75 width cm) with an airflow of 30 cm/s was used to study moth responses to olfactory stimuli. Moths were tested in their scotophase from 17:00 to 00:00 (night hours) at 27 ± 2 °C, 55 ± 5% RH and under 0.3 lux fluorescent lights. Filter paper (Whatman # 1 ® 2 V, Merck KGaA, Darmstadt, Germany) was placed on the Petri plate affixed to a metal stand at the upwind end of the wind tunnel with a 1 mL test sample poured on it. As treatments, various doses of a sex pheromone blend were used, and a commercial lure was simply placed as a rubber septa (Table 1).

A male was released downwind into the wind tunnel and tested for 5 minutes in wind tunnel bioassays. After each test, 5 minutes of clean air was delivered into the wind tunnel. Moth response to the stimulus was recorded as (take-off, 150 cm from the source, 100 cm from the source, or halfway across the wind tunnel, 50 cm from the source, landing on the source). At least 40 virgin males were tested for each treatment. Males were only used once and then discarded.

Table 1: Treatments used for dose optimization for male *S. Frugiperda* moths.

Treatment no.	Abbreviations	Treatments
T1	CSP	Rubber septa from Pest Control India
T2	50 ppm	50 ppm of Sex pheromone Blend
T3	100 ppm	100 ppm of Sex pheromone Blend
T4	150 ppm	150 ppm of Sex pheromone Blend
T5	200 ppm	200 ppm of Sex pheromone Blend
T6	250 ppm	250 ppm of Sex pheromone Blend
T7	300 ppm	300 ppm of Sex pheromone Blend
T8	350 ppm	350 ppm of Sex pheromone Blend
T9	400 ppm	400 ppm of Sex pheromone Blend
T10	Control	Hexane

Note: Sex pheromone blend (Z9-14:OAc: Z7-12:OAc: Z9-11:OAc in the ratio of 85:10:5)

RESULTS AND DISCUSSION

In wind tunnel experiments, the sequential behavioural response of virgin male *S. frugiperda* moths to varying doses (50–400 ppm) of sex pheromone blends compared to a commercial sex pheromone lure showed a significant difference (Table 2). Maximum responses to a sex pheromone lure were seen during the initial flight response (TO – take off), with around 90–100% of males initiating flight from the downwind end. When moths were around 150 cm from the source, there was a significant difference in flight response between the dose levels of 350 ppm (χ^2 = 4.33, P = 0.037) and 400 ppm (χ^2 = 4.72, P = 0.029). A considerable fluctuation between doses was recorded when moths were 100 cm away from the source, with a rather significant difference between 100 ppm (χ^2 = 7.60, P = 0.005) and 250 ppm (χ^2 = 6.77, P = 0.009). The fluctuations in in-flight response among the varying doses were considerably less when moths approached the source at 50 cm. There was a significant difference in 100 ppm (χ^2 = 5.09, P = 0.024), 200 ppm (χ^2 = 5.06, P = 0.024) and 300 ppm (χ^2 = 4.08, P = 0.043). However, only half of the population landed on the source, with the highest 59% of moths landing on the source at 100 ppm and the lowest 39% landing on the source at 50 ppm. Furthermore, behavioural landing on source response varied significantly at 100 ppm (χ^2 = 7.68, P = 0.005) and 150 ppm (χ^2 = 4.32, P = 0.037).

Table 2: Behavioral response of male *S. frugiperda* moths towards varying concentrations of sex pheromone blend in the wind tunnel.

	Virgin male response in percentage (%)					
Lure	**Number of moths tested**	**TO**	**150 cm**	**100 cm**	**50 cm**	**LS**
CSP	48	89.58	72.92	54.17	50.00	41.67
50 ppm	41	90.24	78.05	43.90	43.90	39.02
100 ppm	47	95.74	89.36	74.47 **	65.96 *	59.57 **
150 ppm	49	89.80	83.67	65.31	61.22	55.10 *
200 ppm	44	93.18	84.09	70.45 *	65.91 *	50.00
250 ppm	45	95.56	86.67	73.33 **	51.11	51.11
300 ppm	42	92.86	78.57	69.05 *	64.29 *	50.00
350 ppm	43	100.00	90.70 *	69.77 *	53.49	51.16
400 ppm	47	97.87	91.49 *	70.21 *	55.32	48.94
Control	44	11.36 ***	9.09 ***	2.27 ***	0.00 ***	0.00 ***

Note: Asterisks indicate the difference between the CSP (Commercial sex pheromone) and the varying concentrations of sex pheromone blend used in a behavioral event (χ^2 test; P<0.05 = *, P <0.01 = **, P <0.001 = ***); TO = take-off, 150 cm = 150cm from the source, 100 cm = Halfway to the source, 50 cm = 50cm from the source, LS = Landing on source.

Insect olfaction is essential in several vital processes, including mating, oviposition, and dietary preferences (He *et al*., 2022; Hildebrand and Shepherd 1997). Female moths secrete a species-specific sex pheromone in a specialised gland at the tip of their abdomen, attracting males of the same species (Percy-Cunningham and Macdonald 1987). Thus, the production of sex pheromone compounds in a precise ratio attracts male *S. frugiperda* moths.

Combining three sex pheromone compounds ((Z)-9-14: Ac, (Z)-9-12: Ac, and (Z)-7-12: Ac) in an 85:5:10 ratio produced a better response than commercial sex pheromone lures, which typically function on a single pheromone complex. The composition and the relative ratio of the blend components are species-specific and, when blended with the subsequently specified receptors, play a vital role in species attraction, permitting divergence in mate preferences for newer pheromone blends (Cande *et al*., 2013; Smadja and Butlin 2009).

The moth's response to sex pheromone was observed to be dose-dependent. However, a drop in moth landings was noticed after increasing the dosage to a certain level during the wind tunnel experiments (Fig. 1). This drop might be attributed to the rapid dispersion of pheromones in the environment, which hinders males' seeking abilities. To test this assumption, it was observed that increasing the dose resulted in a significant difference during the initial short flight, implying that males were unable to determine the origin of the source. However, a rise in pheromone levels may behave as an antagonist (Quero *et al*., 1995; Wang *et al*., 2022).

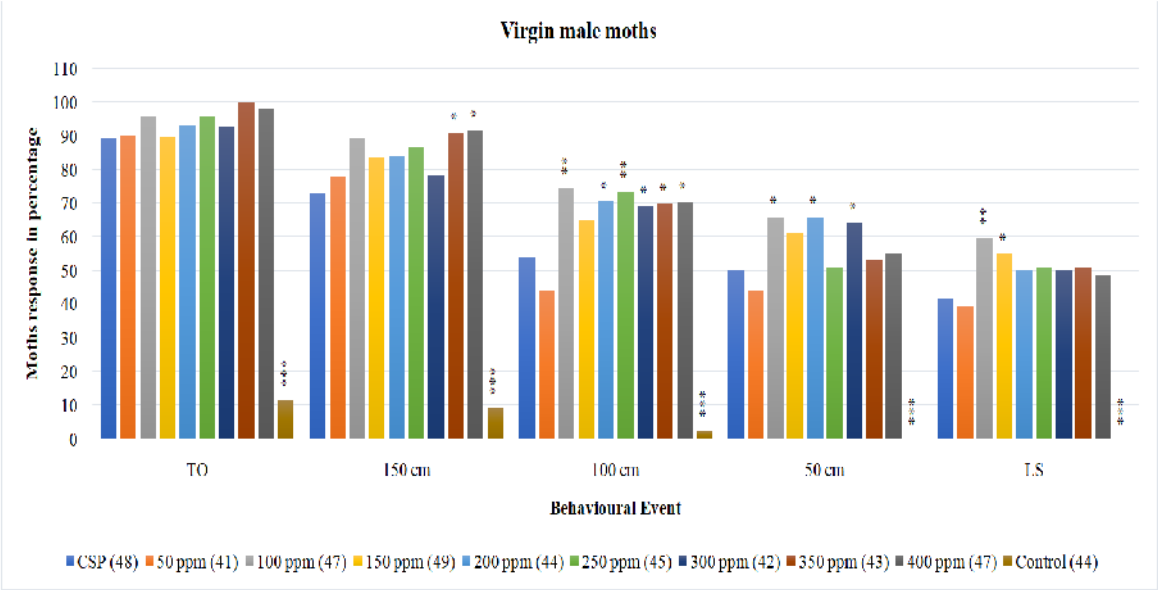
In previous olfactometer research (Gargi, 2021), it was observed that males were attracted to the sex pheromone blend at a concentration of 10 ppm. However, when tested again in a wind tunnel, the concentration jumped to 100ppm, which was precisely ten times higher than in the olfactometer studies. As a result, it can be inferred that the concentration of sex pheromones is significantly affected by the size of the background environment; the larger the size, the higher the concentration. Moreover, when this blend will be taken for field trials, the concentration will change, and the dose needs to be optimised again.

Statistical Analysis. The behavioural response of virgin male *S. frugiperda* moths to different dosages of sex pheromone blend was studied using the χ^2 test (Table 3).

Table 3: Chi-square analysis of the behavioural response of male *S. frugiperda* moths towards the varying concentrations of sex pheromone blend.

Lure	**TO**			**150 cm**			**100 cm**			**50 cm**			**LS**		
	χ^2	***df***	**p-value**	χ^2	***df***	**p-value**	χ^2	***df***	**p-value**	χ^2	***df***	**p-value**	χ^2	***df***	**p-value**
50 ppm	0.00486	1	0.94441	0.36090	1	0.54801	1.94707	1	0.16290	0.74420	1	0.38832	0.16853	1	0.68142
100 ppm	0.42359	1	0.51515	3.70644	1	0.05420	7.60735	1	0.00581	5.09443	1	0.02400	7.68922	1	0.00556
150 ppm	0.00054	1	0.98146	1.58478	1	0.20807	2.29093	1	0.13013	2.51777	1	0.11257	4.32841	1	0.03748
200 ppm	0.14468	1	0.70368	1.71104	1	0.19085	4.89272	1	0.02697	5.06256	1	0.02445	1.66520	1	0.19690
250 ppm	0.39920	1	0.52750	2.59274	1	0.10735	6.77692	1	0.00923	0.02464	1	0.87526	2.13856	1	0.14364
300 ppm	0.12010	1	0.72893	0.43777	1	0.50820	4.08740	1	0.04320	4.08408	1	0.04329	1.66520	1	0.19690
350 ppm	1.21206	1	0.27092	4.33528	1	0.03733	4.49252	1	0.03404	0.24360	1	0.62162	2.16127	1	0.14153
400 ppm	0.76718	1	0.38109	4.72909	1	0.02966	4.74952	1	0.02931	0.56605	1	0.45183	1.26837	1	0.26007
Control	68.30061	1	0	55.87313	1	0	49.72512	1	0	50.00000	1	0	41.67000	1	0

TO = take-off, 150 cm = 150cm from the source, 100 cm = Halfway to the source, 50 cm = 50cm from the source, LS = Landing on source.



The numbers in brackets represent the number of moths utilised in that treatment. Asterisks indicate the difference between the CSP (Commercial sex pheromone) and the varying concentrations of sex pheromone blend used in a behavioral event (χ^2 test; P<0.05 = *, P <0.01 = **, P <0.001 = ***); TO = take-off, 150 cm = 150cm from the source, 100 cm = Halfway to the source, 50 cm = 50cm from the source, LS = Landing on source.

Fig. 1. Behavioral response of male *S. frugiperda* moths towards varying concentrations of sex pheromone blend in the wind tunnel

CONCLUSION

We tested various dosages of sex pheromone blend in wind tunnel studies, and the dosage concentration of 100 ppm was shown to be the most suitable. Furthermore, because various environmental factors are involved in determining the dose for carrying out this experiment, this approach might be utilised as a standard guideline, which is suggested for every researcher to follow in order to provide authenticity to their research.

FUTURE SCOPE

When any further study is done on this end, the results are more likely to vary because when odour blends evolve, the efficacy of male-female communication becomes compromised, unless the preference for novel blends also evolves (Rabhi *et al*., 2014; Wang *et al*., 2022). So, when working with synthetic chemical compounds for research purposes, dosage optimization becomes essential to offer accurate data.

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Conflict of Interests. None.

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