



Mass trapping using sex pheromone for the management of diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in cabbage

VINAY KUMAR THIRUMALAHATTI MUNIKRISHNAPPA^{1,2}, KESAVAN SUBAHARAN^{1*}, RAJASHEKHARAI AH VANI², MARKANDEYA GORANTLA³, AMALA UDAYAKUMAR¹, SUJITHRA MARUTHAKASI¹ and SATYA NAND SUSHIL¹

¹ICAR - National Bureau of Agricultural Insect Resources, H A Farm Post, Bellary Road, Hebbal, Bengaluru 560024, Karnataka, India.

²Department of Biotechnology and Genetics, Jain (Deemed-to-be University), Bengaluru 560027, Karnataka, India.

³ATGC Biotech Pvt. Ltd., Hyderabad 500014, Telangana, India

*E-mail: kesavan.subaharan@icar.org.in

ABSTRACT: The diamondback moth (DBM) *Plutella xylostella* (Lepidoptera: Plutellidae), is a major pest of cruciferous crops cause severe economic losses worldwide. Continued dependence on chemical insecticides has led to development of resistance, negative impact on non-targets as well as environmental pollution. These problems prompt a shift towards behaviour-based pest management strategies. This study evaluated the electrophysiological and behavioural responses of *P. xylostella* to synthetic sex pheromone blends of Z-11-hexadecenyl acetate and Z-11-hexadecenal formulated in different ratios and loaded onto rubber septa. The PH blend (49.5:49.5:1) elicited the highest antennal response (~3.3 mV) and attracted the most moths in behavioural assays, followed by the (10:10:0.1) (~2.6 mV) and (7:3:1) (~1.9 mV) blends. Field trapping trials confirmed that the PH blend captured the highest number of moths (~847.2) among all tested formulations. These findings provide insights for optimizing pheromone blends for effective mass trapping and monitoring of *P. xylostella*, supporting sustainable and eco-friendly integrated pest management strategies

Keywords: *Plutella xylostella*; Sex pheromone; Electroantennography; Z-11-hexadecenal; White sticky traps

INTRODUCTION

Among the major pests of brassicaceous crops, the diamondback moth (DBM), *Plutella xylostella* is a key pest capable of completing 10 to 14 generations per year, by inflicting losses up to 90% in yield (Furlong *et al.*, 2013). Its host range and ability to thrive across a wide range of temperatures and resistance against insecticide make it challenging to manage DBM (Furlong *et al.*, 2013; Mallot *et al.*, 2019). Global cost of crop protection measures to manage this pest is estimated to be US\$ 4–5 billion (Zalucki *et al.*, 2012). Though chemical control is a commonly adopted method to manage *P. xylostella* it has developed resistance to almost all insecticides that are commonly used for its control (Tamilselvan *et al.*, 2021; Mota-Sanchez and Wise, 2025). Insecticides used to manage *P. xylostella* provide a varying degree of success (Pudasaini *et al.*, 2022; Sun *et al.*, 2022; Opoloioi *et al.*, 2024). Intensive selection pressure has developed population that can withstand the field dosages through a combination of degrading enzymes and their association with symbiotic bacteria like *Enterococcus mundtii* that metabolise organophosphates (Li *et al.*,

2025) underscored the need for developing alternative strategies that exploit the insects behaviour (Gregg *et al.*, 2018; Blassioli-Moraes *et al.*, 2019).

In recent years, pest management strategies have increasingly adopted the chemo-ecological approach utilizing semiochemicals, providing an environmentally friendly alternative to conventional control methods (Reddy and Guerrero, 2010; Witzgall *et al.*, 2010). Unlike pesticides that require broad application across fields, this modern approach uses semiochemical lures placed at specific sites for pest monitoring and mass trapping, thereby minimizing environmental impact (Reddy and Guerrero, 2010). A major advantage of pheromone-based pest management is its high specificity typically attracting only the target pest species thus significantly reducing the risk to non-target organisms in the ecosystem (Reddy and Guerrero, 2010; Vacas *et al.*, 2012; Gregg *et al.*, 2018; Galli *et al.*, 2024).

Synthetic sex pheromone lures are widely employed for monitoring and mass trapping of adult male *P. xylostella*. However, the practical challenge in using

pheromone-based techniques lies in the geographic variability of insect responses to different ratios of pheromone components. Populations of the diamondback moth (DBM) exhibit region-specific variations in their responses to different pheromone blends, indicating differential sensitivity to the major components within blends (Xie *et al.*, 2010; Li *et al.*, 2011; Liu *et al.*, 2011). The male *P. xylostella* exhibit the strongest response to blends containing Z-11-hexadecenyl acetate and Z-11-hexadecenal as the primary constituents (Reddy and Urs, 1996; Reddy and Guerrero, 2000; Topagiet *et al.*, 2018). Consequently, various sex pheromone formulations were developed and assessed as an eco-friendly strategy for *P. xylostella* management. In the present study, the pheromone blends were assessed for their physiological and behavioural response and the blends were impregnated onto rubber septa and evaluated for field trapping efficiency.

MATERIALS AND METHODS

Materials used

The sex pheromone components of *P. xylostella*, (Z)-11-Hexadecenal (Z11-16: Ald), (Z)-11-Hexadecenyl acetate (Z11-16: Ac), and (Z)-11-Hexadecen-1-ol (Z11-16: OH), were supplied by ATGC Biotech Pvt. Ltd., Hyderabad. Dichloromethane (DCM) (HPLC grade), butylated hydroxytoluene (BHT) were sourced from Sigma Aldrich.

Preparation of pheromonecomposites

The pheromone blend of *P. xylostella*, consisting of Z-11-hexadecenal, Z-11-hexadecenyl acetate, and Z-11-hexadecen-1-ol in varying ratios, was diluted with dichloromethane (DCM) at a 2:30 proportion. The diluted mixture was then applied onto rubber septa, which were air-dried to allow complete solvent evaporation before being sealed as lures.

Rearing of diamondback moth *P. xylostella*

Larvae of the diamondback moth (DBM) were collected from agricultural fields in Dinneri Harohalli, Kolar district, Karnataka, India (12°57'01.3"N, 78°00'02.6"E). Morphological and molecular identification of the specimens was conducted at ICAR–NBAIR. Larvae from the second to fourth instar stages were reared in aluminium cages (30 × 30 × 30 cm) on fresh cabbage leaves, which were replaced every two days until pupation. Pupae were then transferred

to separate cages to allow adult emergence. Mustard seedlings served as oviposition substrates, and a 10% sugar solution was provided as an adult food source. Eggs laid on the leaves were collected daily and transferred to culture containers for colony maintenance. The insect culture was maintained under controlled laboratory conditions at 26 ± 2°C, 75 ± 5% relative humidity, and a 12:12 h light–dark cycle. Various developmental stages from this laboratory colony were utilized for subsequent experimental studies.

Electroantennography (EAG)

The antennal responses of unmated and mated male *P. xylostella* adults to various sex pheromone blends were recorded using an electroantennographic (EAG) system (Syntech). A dual-electrode probe was used to mount the antennae, which were carefully excised from the heads of adult moths. The basal portion containing the scape was connected to the ground electrode, while the distal tip was attached to the recording electrode using conductive gel (Spectra 360 Parker, Orange, New Jersey). A continuous stream of clean, charcoal-filtered air was directed over the antennae through a delivery tube positioned 15 mm away.

The pheromone components of *P. xylostella* (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, and (Z)-11-hexadecen-1-ol were prepared in different ratios and diluted in HPLC-grade dichloromethane (DCM) to a concentration of 1 µg/µl. One microliter of this solution (equivalent to 1 µg pheromone) was applied onto Whatman filter paper strips (2 cm × 4 mm; Advantec 5C, Japan), air-dried for 1 min in a fume hood, and inserted into Pasteur pipettes. These were connected to a stimulus controller (CS-05 Syntech) via Tygon silicone tubing. Thirty seconds after placing the filter paper inside the pipette, the first puff was released. After another 30s, the mounted antennae were exposed to the vapor phase of the stimulus through the delivery tube positioned 15 mm upstream, under a continuous airflow (pulse duration: 0.5 s; continuous flow: 25 ml/s; pulse flow: 21 ml/s), following the method of Senthoooraja *et al.* (2021). A 20 s interval was maintained between successive stimuli.

Antennal signals were captured through a high-impedance probe connected to an amplifier (IDAC-4, Syntech), and responses were recorded using EAG software (Syntech). The results were expressed as the summed neuronal responses, sorted by shape and amplitude, for 1s following stimulus onset. Control

stimuli were applied at the beginning, middle, and end of each recording session (Venugopal & Subaharan, 2019; Kaur *et al.*, 2021). Each pheromone blend was tested on ten antennae, with three replications per stimulus per antenna in a randomized design. To eliminate solvent and background effects, EAG responses were corrected by subtracting the control readings recorded before and after each replication, following the approach of Kaur *et al.* (2021).

Y-tube olfactometer assay

The olfactory response of *P. xylostella* adult males to different sex pheromone blends were evaluated using a glass Y-tube olfactometer having the main arm of 17 cm and choice arms of 17 cm with an inner diameter of 4 cm. Atmospheric air pumped using an air sampler was allowed to flow out through activated charcoal cartridge with a flow rate of 0.5 l/min. The purified air is then let into the arms of the Y-tube with a steady flow rate (Fig. 1). Whatman No. 1 filter paper stripes (3 cm in length and 0.5 cm in width) were treated with 10 μ l of 100 ppm of compounds diluted in dichloromethane was loaded, and the paper strips were allowed to dry at room temperature for 1 min to permit the solvent to evaporate. The paper strips prepared as mentioned above, without the odorants were used as control. Both treated and control paper strips were inserted into an odor tube that is connected between the airflow tube and the Y-tube arm. The entire Y-tube setup was placed inclined at 20°. A pairwise comparison was made between odorants and control. The insects starved for 4 h with water satiation before the test. Both unmated and mated *P. xylostella* male moths were individually introduced into the main arm. The choice made by the moths in an arm was considered if they crossed the halfway mark made in the arm within 3 min of the start of the test. Those insects that failed to participate in the test were considered non-respondents. The odorants were switched between the arms to avoid the position effect. The test was conducted at a room temperature of 25 ± 2 °C under red light with slight modification as suggested by (Ravindran *et al.*, 2019; Senthoooraja *et al.*, 2021). Four sets of ten insects per replicate were used to assess the olfactory response.

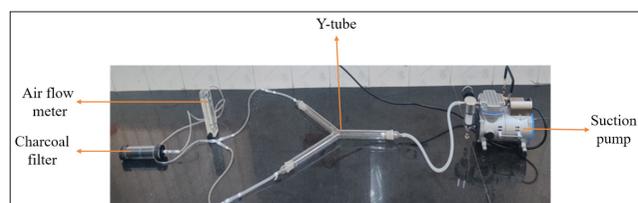


Fig.1. Y-tube olfactometer setup

Assessing the dissipation of pheromone

A dissipation study was conducted to determine the release rate of pheromone-loaded rubber septa over an extended period. The formulation was prepared by combining 2 μ L of pheromone and 0.25 mg of butylated hydroxytoluene (BHT) in 30 μ L of dichloromethane (DCM), which was then loaded onto rubber septa and air-dried. The pheromone lures were stored at room temperature with measures taken to minimize temperature fluctuations. The entire setup was maintained under a fume hood, and weight loss was monitored gravimetrically using a precision balance (Shimadzu) for 60 days, beginning from day zero of the experiment, following the method described by Ravindran *et al.* (2019). Each formulation was tested with ten replicates.

Assessing the field efficacy of the pheromone blend loaded in rubber septa

The field performance of rubber septa loaded with the *P. xylostella* pheromone blend was assessed at Kolar, Karnataka, India (12°95'93.79"N, 78°00'33.71"E) from November 2023 to January 2024. The formulation was prepared by mixing 2 μ L of pheromone and 0.25 mg of butylated hydroxytoluene (BHT) in 30 μ L of dichloromethane (DCM), which was then applied onto rubber septa. After complete evaporation of the solvent, the lures were sealed. These prepared lures were placed in white delta sticky traps (18 cm \times 10 cm) positioned 2 feet above the ground to capture adult moths. Sticky traps without pheromone and traps containing blank rubber septa served as controls.

The experiment was conducted using a completely randomized block design with five replicates. Moth catches were recorded weekly, and the sticky traps were replaced with fresh ones after each count. Within each block, traps were spaced 5 meters apart, while the distance between blocks was approximately 25 meters. Trap positions within each block were re-randomized after every observation. New delta sticky traps were used until the trap catch levels declined significantly. The initial observation was made 24 hours after trap deployment.

Statistical analysis

The response of moths to Y-tube choice assay was subjected to Chi-squared test. The EAG responses and field trapping data were subjected to One-way ANOVA, followed by Tukey's HSD to calculate the differences in significance of the data at $p \leq 0.05$.

RESULTS AND DISCUSSION

Electrophysiological response of unmated male *P. xylostella* to the sex pheromone

The study revealed that 3-day-old unmated male *P. xylostella* displayed differential antennal response to sex pheromone constituents, with (Z)-11-hexadecenal (Z-11-16: Ald) causing highest amplitude response (~3.8 mV), followed by (Z)-11-hexadecenyl acetate (Z-11-16: Ac) (~3.2 mV), and (Z)-11-hexadecen-1-ol (Z-11-16: OH) (~1.7 mV), respectively (Fig. 2). These results corroborate with previous reports on establishing (Z-11-16: Ald) and (Z-11-16: Ac) as the major constituent of *P. xylostella* pheromone (Koshihara, 1985; Lee *et al.*, 2005). The observed antennal response pattern in our study aligns with earlier findings indicating that male antennae exhibit heightened sensitivity to both aldehyde and acetate components, which constitute the predominant elements in female pheromone glands (Lee *et al.*, 2005; Dai *et al.*, 2016).

The electroantennogram (EAG) response to (Z-11-16: Ald) observed reinforces the conclusions of (Wu *et al.*, 2020), who indicated that antennal reactions to this aldehyde component remained consistent across various adult ages in *P. xylostella* confirming its role as primary cue for mate recognition throughout the adult male lifespan. The intermediate response to (Z-11-16: Ac) (~3.2 mV) is particularly significant, as (Wu *et al.*, 2020) illustrated that sensitivity to this acetate component demonstrates age-dependent plasticity, characterized by substantial fluctuations in EAG responsiveness. The selection of 3-day-old males in our study may signify a developmental phase wherein acetate sensitivity is especially pertinent for mate-finding behavior. Findings of this study reveal pheromone blends elicited stronger antennal responses than any individual component tested. The 49.5:49.5:1 blend caused highest response (~4.5 mV), exceedingly over 18% than the response for the major compound. This confirms the *P. xylostella* antennae tuned to detect particular ratios of pheromone components rather than responding to individual compounds in isolation, a phenomenon that is reported in lepidopteran pheromone communication systems.

The physiological response of the 49.5:49.5:1 blend (Ald:Ac:OH) is intriguing when compared to the 8:3:2 ratio observed from *P. xylostella* gland as reported by (Lee *et al.*, 2005). Although our optimized ratio differs substantially from the natural proportion, it may represent a better stimulus that maximizes antennal receptor activation. The regional variation in optimal blend ratios

for *P. xylostella* attraction in the Pearl River Delta, where formulations such as 30:70:0.1 and 50:50 produced enhanced trap catches compared to traditional ratios (Dai *et al.*, 2016). Our observation that a near-equimolar blend of the two major components with trace amounts of the alcohol produces maximal EAG responses lends support to the idea that blend optimization can exceed natural ratios in stimulating peripheral sensory neurons.

The 10:10:0.1 blend also elicited a strong response (~4.2 mV), suggesting that absolute component quantities may be less critical than maintaining appropriate ratios, at least within a concentration range. Interestingly, the 7:3:1 blend (~3.5 mV), which is close to natural gland ratio (Lee *et al.*, 2005), produced a moderate response that was still substantially higher than any single component alone. Though natural pheromone ratios are biologically relevant, the antennal detection system exhibits flexibility in responding to ratio variations, possibly reflecting population-level variation or adaptation to different environmental contexts (Dai *et al.*, 2016). The synergistic response observed with multi-component blends reflects the simultaneous activation of olfactory receptor neuron (ORN) classes tuned to pheromone components. The integration of signals from multiple ORN classes at the antennal lobe level may produce enhanced neural output compared to activation of a single receptor type, thereby resulting in the amplified EAG responses we observed with blends in this study. Three-day old adults were considered for assessing the pheromone response as few components like Z-11-16: Ald remained consistent regardless of age, their sensitivity to Z-11-16: Ac changed significantly as they matured. Hence, by using the 3-day-old males, for electrophysiological assays helps to capture them at their peak responsiveness.

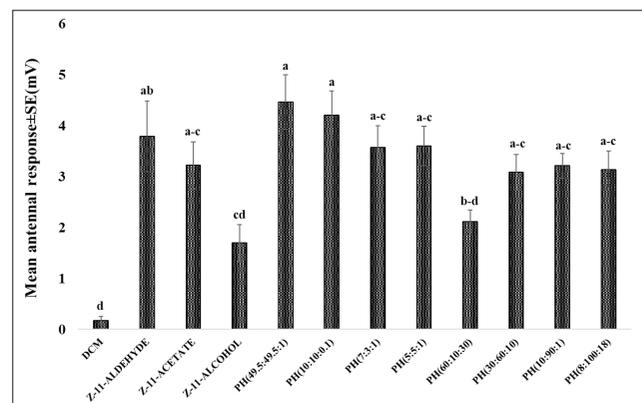


Fig. 2. EAG response of adult unmated male *P. xylostella* to sex pheromone individual components and different blend ratios in response to solvent. n=10, Bars followed by the same letter are not significantly different ($p \leq 0.05$). Results are represented as mean \pm S.E.

Electrophysiological response of adult male *P. xylostella* to pheromone blends released from rubber septa

The electrophysiological responses of adult unmated male *P. xylostella* were assessed to verify whether the pheromone blend loaded onto rubber septa was released in a form capable of eliciting antennal neuronal activity. Male antennae were exposed to pheromone-loaded septa, and the recorded responses were analysed to determine the efficiency of pheromone emission. Antennal responses were measured for three treatments: pheromone blend (PH) (49.5:49.5:1) + septa, blend (10:10:0.1) + septa, and PH (7:3:1) + septa. The highest response (~3.3 mV) was obtained with PH (49.5:49.5:1) + septa, followed by PH (10:10:0.1) + septa (~2.6 mV) and PH (7:3:1) + septa (~1.9 mV) (Fig. 3). These results suggest that the pheromone released from PH (49.5:49.5:1) + septa maintained an optimal ratio for effective neuronal stimulation. In contrast, control treatments with dichloromethane (~0.2 mV) and blank rubber septa (~0.1 mV) elicited only minimal responses (Fig. 3). At 3 days post-emergence, males are typically sexually mature and actively engaged in mate-seeking behaviour, representing an optimal stage for assessing pheromone sensitivity (Wu *et al.*, 2020). The unmated status of test males is also relevant, as mating experience can alter peripheral and central olfactory processing in some insect species, though specific data on mating-status effects in *P. xylostella* males remain limited in the literature.

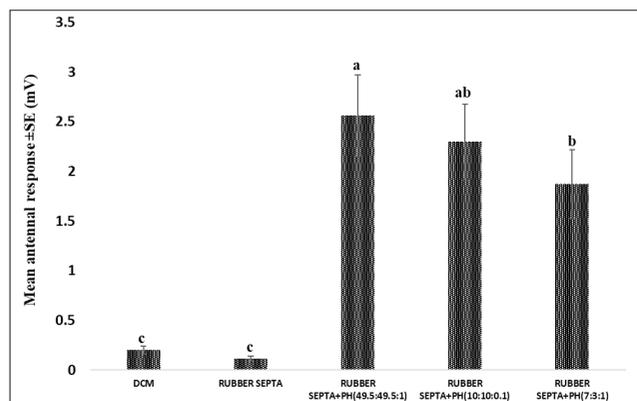


Fig. 3. EAG response of adult unmated male *P. xylostella* to different pheromone blend ratios in dispenser in response to solvent. $n=10$, Bars followed by the same letter are not significantly different ($p \leq 0.05$). Results are represented as mean \pm S.E.

Y-tube olfactometer

Behavioural Responses of adult males of *P. xylostella* to the sex pheromone in different blends

The behavioural responses of unmated male *P. xylostella* to sex pheromone released from different

dispensers were evaluated. The results showed a significant attraction toward the arms containing PH (49.5:49.5:1) ($p \leq 0.01$), PH (49.5:49.5:1) + septa ($p \leq 0.01$), PH (10:10:0.1) + septa ($p \leq 0.01$), and PH (7:3:1) + septa ($p \leq 0.05$). These responses were significantly greater than those recorded for the control arm containing dichloromethane (DCM) alone. In contrast, no significant response was observed toward the DCM control arm ($p \leq 0.79$) (Fig. 4). Overall, the findings indicate that all tested dispensers efficiently released the pheromone blends, eliciting strong behavioural attraction in unmated males and confirming their suitability for pheromone-based monitoring or control applications.

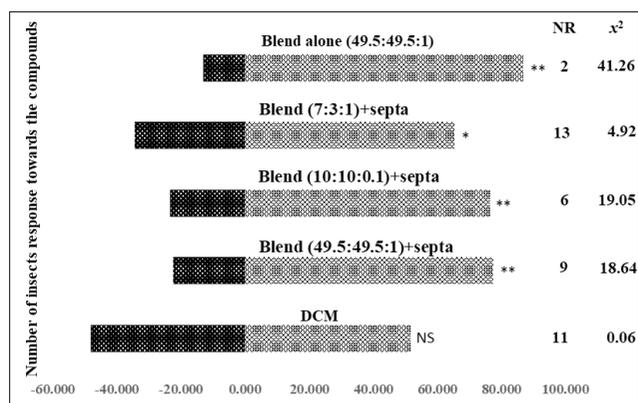


Fig. 4. Behavioural responses of adult unmated males of *P. xylostella* to different blend ratios in response to solvent. NR= Non respondents, $n=10^{-40}$, The insects that failed to respond were excluded from the statistical analysis. An asterisk indicates a statistically significant difference from the control (DCM) in each arm, as determined by the chi-square test ($P \leq 0.01 = **$, $P \leq 0.02 = *$).

The observed strong attraction of unmated male *P. xylostella* to the tested pheromone blends is consistent with the well-established composition of the female sex pheromone, which comprises three primary bioactive components: (Z)-11-hexadecenal (Ald), (Z)-11-hexadecenyl acetate (Ac), and (Z)-11-hexadecen-1-ol (OH) (Suckling *et al.*, 2002; Yang *et al.*, 2007). The acetate component typically dominates in female gland extracts and field-active formulations, with behavioral studies demonstrating that both the aldehyde and acetate are essential for male attraction, while small amounts of the alcohol can modulate attractiveness (Yang *et al.*, 2007). The three pheromone blends tested in this study—PH (49.5:49.5:1), PH (10:10:0.1), and PH (7:3:1) represent variations in the relative proportions of these key components, all of which elicited significant male responses.

Dissipation studies of pheromone loaded in rubber septa

The dissipation pattern of the sex pheromone blend (49.5:49.5:1) was analysed by loading it onto rubber septa to evaluate their shelf-life capacity. The sex pheromone alone dissipated within 15 days. In contrast, the sex pheromone loaded onto the rubber septa lasted 45 days, as shown in (Fig. 5).

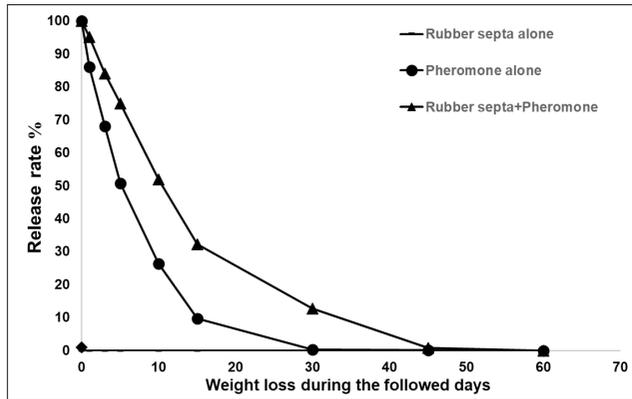


Fig. 5. Dissipation pattern of rubber septa loaded with sex pheromone blend for shelf-life study n=10. Values are represented as mean per cent release

The effectiveness of all tested dispensers in releasing pheromone blends and eliciting male attraction underscores the importance of appropriate dispenser selection for pheromone-based applications. Rubber septa have been widely used as pheromone

dispensers due to their simplicity, cost-effectiveness, and proven field efficacy (Chisholm *et al.*, 1979; Mottus *et al.*, 2004; Tacain *et al.*, 2016) conducted comprehensive optimization studies comparing various dispenser types for *P. xylostella*, including gray and red rubber septa, mini dispensers, and novel matrices. Their findings indicated that gray rubber septa captured more males than red septa in canola field trials, and that dispenser-specific release rates significantly influenced trap catches and monitoring duration (Mottus *et al.*, 2004). The present study's use of septa combined with different pheromone ratios confirms that this dispenser format provides consistent pheromone release suitable for behavioral bioassays and field monitoring.

Assessing the field efficacy of the pheromone blend loaded in the rubber septa

The weekly observations demonstrated clear differences in the insect trapping efficacy among the tested blends. Control traps, which lacked any pheromone baiting, showed no significant insect captures throughout the study period. Traps baited with PH (49.5:49.5:1) + septa and PH (10:10:0.1) + septa, began capturing insects effectively till 6th week, traps with the PH (49.5:49.5:1) + septa captured a greater number of male DBM moths compared to traps with the PH (10:10:0.1) + septa lure (~847.2 vs. 711.4 males per trap; Table.1).

Table.1 Dosage of sex pheromone in rubber septa (2 mg) and *P. xylostella* moth capture.

Pheromone Dispenser	Mean (±SE) number of male moths captured/trap/week								Total moths captured per trap
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	
Control	11 ± 1.37 ^b	11.2 ± 1.93 ^b	2.2 ± 0.37 ^b	1.8 ± 0.37 ^b	0.4 ± 0.24 ^b	0.2 ± 0.2 ^c	1.6 ± 0.4 ^b	2.2 ± 0.37 ^b	30.6
Rubber septa alone	9.8 ± 1.24 ^b	10.4 ± 0.92 ^b	3 ± 0.7 ^b	1.4 ± 0.5 ^b	0.4 ± 0.24 ^b	0.2 ± 0.2 ^c	1.2 ± 0.37 ^b	1.6 ± 0.4 ^b	28
Rubber septa +blend (10:10:0.1)	192.6 ± 21.08 ^a	309.4 ± 27.33 ^a	113.4 ± 12.8 ^a	54.2 ± 4.38 ^a	28 ± 9.03 ^a	8 ± 0.94 ^b	3 ± 0.7 ^b	2.8 ± 0.58 ^b	711.4
Rubber septa +blend (49.5:49.5:1)	221.2 ± 20.99 ^a	323.8 ± 37.37 ^a	147.4 ± 19.8 ^a	68.4 ± 5.87 ^a	42.8 ± 8.1 ^a	16.6 ± 2.2 ^a	16.6 ± 2 ^a	10.4 ± 1.9 ^a	847.2

N=16, Means (±SE). In column means followed by the same alphabet do not differ significantly Tukey's HSD *P* < 0.05

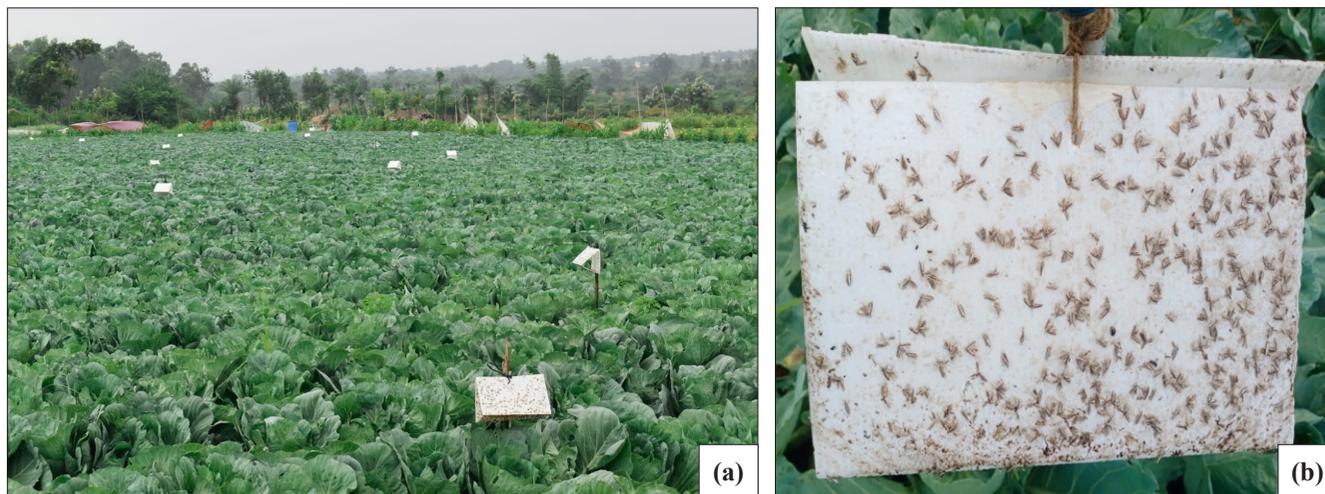


Fig. 6 (a). Photograph showing the view of the experimental field
(b) *P. xylostella* moths attracted to the pheromone blend

The complete absence of captures in unbaited control traps throughout the study period confirms the high specificity of sex pheromone-mediated attraction in *P. xylostella* and validates the experimental design. This finding is consistent with established principles of lepidopteran chemical ecology, where male moths exhibit strong and selective responses to female sex pheromone components (Zahoor *et al.*, 2023; Chi *et al.*, 2024). Previous field studies have consistently demonstrated that synthetic pheromone lures attract male DBM at rates comparable to live unmated females, while non-baited traps fail to intercept significant numbers of adults (Dang *et al.*, 2016; Dai *et al.*, 2016). The species-specific nature of pheromone attraction is a critical advantage for monitoring programs, as it eliminates background noise from non-target insects and provides unambiguous data on target pest populations (González-Fuentes *et al.*, 2023).

Chisholm *et al.* (1979) reported similar results in early field trials, where control traps without pheromone dispensers captured negligible numbers of DBM males, whereas pheromone-baited traps produced substantial catches. This specificity is particularly valuable in diverse agroecosystems where multiple lepidopteran species may be present, as pheromone traps selectively monitor the target pest without interference from non-target organisms. The empty control traps in the present study therefore provide strong evidence that the observed captures in pheromone-baited traps reflect genuine pheromone-mediated attraction rather than non-specific trapping artefacts or environmental factors.

The superior performance of traps baited with PH (49.5:49.5:1) + septa compared to PH (10:10:0.1) +

septa (~847.2 vs. 711.4 males per trap) represents a 19% increase in cumulative trap catches and highlights the importance of pheromone loading and release rate in field trapping efficacy. While both blends maintained the same relative proportions of pheromone components, they differed substantially in absolute loading, with the PH (49.5:49.5:1) formulation containing approximately five times more pheromone per dispenser. This difference in loading translates to higher initial release rates and potentially extended effective field life, both of which can significantly influence trap performance (Shi *et al.*, 2011).

The literature provides substantial evidence that dispenser loading and release rate critically affect field trap catches. Mottus *et al.* (1997) conducted comprehensive optimization studies comparing various pheromone dispensers for *P. xylostella* and found that release rates in the range of 8–17 ng/h from mini dispensers provided optimal trap catches throughout the monitoring season. Their work demonstrated that both under-dosing and over-dosing could reduce trapping efficiency, with under-dosed lures failing to attract males from adequate distances and over-dosed lures potentially causing behavioural disruption or plume-following difficulties (Mottus *et al.*, 1997). The higher loading in the PH (49.5:49.5:1) + septa used in the present study likely positioned this formulation within or closer to the optimal release rate window, explaining its superior field performance.

Geographic and population-specific variation in optimal blend ratios have been documented extensively. Yang *et al.* (2007) reported that Korean populations of *P. xylostella* responded most strongly to ternary blends with specific component proportions (10:90:1

Ald:Ac:OH), while Dai *et al.* (2016) found that different blend formulations performed optimally in the Pearl River Delta region of China. However, these studies also emphasized that absolute release rate, not just component proportions, determines field efficacy. The present results align with this principle: given identical component proportions in both tested blends, the difference in trap catches can be attributed primarily to the difference in pheromone loading and consequent release kinetics.

CONCLUSION

Irreversible damage caused by pesticide-based crop protection motivates shift to eco-friendly alternatives, such as the chemo-ecological approach involving pheromone-assisted insect monitoring and mass trapping. However, the geographic variation in insect responses to different blend ratios of the pheromone components poses a challenge to achieving effective pest control. To overcome this limitation, different sex pheromone blends were prepared and tested for the management of *P. xylostella* pheromone as an eco-friendly approach to pest management. This improvement significantly enhanced the field efficacy of the rubber septa lures loaded with *P. xylostella* pheromone. Further, there is considerable potential to expand this work to include different pheromone types.

AUTHOR CONTRIBUTIONS

VKT and KS conceived and designed the experiments. All authors performed experiments and collected data. VKT, KS, and VR conducted statistical analyses. VKT and KS performed electrophysiology, behavioral and field experiments. All authors interpreted and discussed results. VKT and KS wrote the manuscript with inputs from all the authors; KS supervised the research.

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