Journal of Animal & Plant Sciences, 34(5): 2024, Page: 1127-1138
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.5.0795

OPTIMIZATION OF SEX PHEROMONE DOSE AND TRAP DENSITY FOR MATING DISRUPTION OF *CHILO SUPPRESSALIS* (LEPIDOPTERA; PYRILADAE)

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ABSTRACT

The rice stem borer, *Chilo suppressalis* (Walker) is an oligophagous pest that mainly feeds on rice and currently is largely controlled through chemicals, leading to several ecological problems and often low control efficiency due to the larval feeding habits. Therefore, the alternative and eco-friendly control strategies is needed to manage this noxious insect pests, applications of trapping devices specifically sex pheromone traps are one of the most substitute control methods to monitoring and control moths by killing or mating disruption on large scale. The aim of the current study was to optimize the appropriate pheromone dose, trap density and trap placement height for mating disruption of *C. suppressalis*. During first year (2021), a mixture of two pheromones (Z11-16: Ald and Z9-16: Ald) in equal ratio (50:50) were applied at different doses (50, 100, 200, 300 and 500µg/20µL), with various trap heights (3, 4.5 and 6 feet) and compared with insecticide (fipronil G 0.2% 15 kg/ha). Results revealed that, 200 and 300µg/20µL caught significantly maximum (119.63±15.86) moths of *C. suppressalis* and resulted in lowest (4.96%) damage (white ear and dead hearts) as compared to other doses and treatments. The second year´s trial was designed to optimize the trap density in which 8, 12, 18 and 25 traps/ha were tested with a median dosage 250µg/20µl, based on first year's results. It exhibited that 18 traps/ha captured maximum number (121.95±19.77) moths and reduced the damage symptoms significantly followed by other 25, 12 and 8 traps/ha with significant difference ($P \leq 0.001$). Moreover, results of best treatment (appropriate sex pheromone dosage, trap placement height and trap density/ha) revealed highest (3:52) cost benefit ratio with minimum (7.90 $) expenditure also highest yield 7400 kg/ha was recorded as compared to other treatments. Therefore, installation of 18 traps/ha at 4.5 feet height with a dose of 250µg/20µL can manage *C. suppressalis* in rice-growing areas and consequently reduce management cost as compared to the insecticides. Thus, it is recommended as an important component for the eco-friendly management of rice stem borer to ensure food security.

Key words: *Chilo suppressalis*, Pheromone dosage, Trap density,Trap height and mating disruption.

Published first online August 22, 2024 Published final October 22, 2024

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the essential crops and primary food source for more than half of the world's population and particularly in Asia 90% of people depend on rice as staple food (Rahimoon *et al*., 2023; Fukagawa and Ziska, 2019; Nguyen *et al*., 2008). The rice stem borer, *Chilo suppressalis* (Walker) is a serious threat to rice crop which is found almost everywhere in rice growing areas and typically accounted for over 85% of the borer populations in rice crops globally (Nyaupane, 2022), from the seedling stage until crop maturity (Rahimoon *et al*., 2021). Their infection resulted in white ear head during the reproductive stage and dead heart during the tillering stage (Kumar *et al*., 2023), which causes significant damage in every crop season resulting in 60 % economic losses which is major threat to the food security (Baskaran *et al*., 2019; Liang *et al*., 2020; Lou *et al*., 2013). To overcome such a huge loss caused by the rice striped stem borer, several control methods have been practiced with the chemical application to be one of most prominent method (Chen and Klein 2012; Zheng *et al*., 2011). However, the insecticides are not always effective due to the larval feeding habits (Luo *et al*., 2019; Huang *et al*., 2011). In addition, the development of pest resistance, adverse ecological effects and impact on humans health due to insecticide applications has become increasingly prominent now a days (Graham and Peter, 2009; Huang *et al*., 2003). To overcome the population of noxious insect pests, rice growers are frequently applying chemical insecticides (Rahimoon *et al*., 2023), indiscriminate use of chemicals has led environmental pollution, human health effects, insect pests resistant, impacts on wildlife beneficial arthropod species (Shehnaz *et al*., 2023; Su *et al*., 2014). For the management of rice borers and noctuid moths, adoption of eco-friendly strategies is needed which will ultimately reduce the in judicious use of chemicals in the agro-ecosystem (Mandla *et al*., 2024). Communication behavior in pyralid moths is mediated by specific chemical cues such as sex pheromones and host plant volatiles, which are closely associated with feeding and reproduction (Pophof, 2005). Sex pheromone application is becoming a valuable and efficient strategy of biotechnological control to suppress lepidopteran pests (Chen *et al*., 2014). For example, pheromone-baited traps have been widely used in male flight monitoring, population forecasting, mass trapping, and mating disruption to control a lot of lepidopteran pest species (Witzgall *et al*., 2010; Chen *et al*., 2014). Mating disruption technique by using sex pheromones have been documented (Witzgall *et al*., 2010) to disturb the lepidopteran moth's mating behavior (Cork, 2004). The (Z)-11-hexadecenal, (Z)-9-hexadecenal and (Z)-13-octadecenal has been identified as the sex pheromone components of *C. suppressalis* (Tatsuki, 1990), and could significantly enhance the male attractants in field tests (Kondo *et al*., 1993; Cork and Basu, 1996). A limited number of field experiments have been performed to analyze the effect of pheromones as a mating disruption agent for controlling the rice striped stem borer (Alfaro *et al*., 2009; Chen *et al*., 2003). Moreover, a variety of studies suggested the appropriate doses and trap density; for example, Howse *et al*., (1998) recommended (Z11-18: OH) 100 dispensers/ha with 400mg each for controlling the *C. suppressalis*. Further, previous research suggested that 2500 PVC dispensers/ha with 8mg (Casagrande, 1993) 16, 25 and 31 dispensers/ha containing dose 12.4, 10.0 and 6.40g/ha, showed remarkable mating disruption (Alfaro *et al*., 2009), respectively. The main constraint to adopt the mating disruption method for managing lepidopteron moths is the excessive application of pesticides and lack of knowledge to the farmers about proper dosage proper application of integrated pest management (IPM) tools (Pathan *et al*., 2023). Thus, the present research was subjected to determine the effectiveness of sex pheromone traps for attraction, mating disruption and control of rice stem bore under rice field condition, also minimize the application of pesticide. However, based on previous findings the farmers are applying sex pheromone traps but optimum dose, pheromone trap density and appropriate trap placement height are still unclear in large rice-growing areas of Pakistan. Therefore, the current research was conducted to determine the optimal pheromone dose, trap density and appropriate trap placement height for mating disruption of *C. suppressalis.* This research not only provide the essential information to develop effective controlling model against noctuid moths but in addition could reduce the oviposition rate and larval infestation which caused by rice stem borer in ice field and monitoring efficacy of pheromone traps at releasing sites.

MATERIALS AND METHODS

Field trials were conducted to determine the optimal dose of sex pheromone, trap density and trap height for mating disruption for the management of *Chilo suppressalis* in rice-growing areas (Larkana) Sindh, during the 2021-22.

Field lay out and experimental set up: The experiment was designed by randomized complete block design (RCBD) with three replications of each treatment. All the agronomical practices such as land preparation, timely sowing, proper weeding, irrigation, and fertilizer applications were carried out according to the need of crops. The different doses with three replicates of each were tested with various trap heights, i.e, 3, 4.5 and 6 feet. Sex pheromone traps were installed with the support of wooden stands and treatment plots had never been treated with any other control method. There were two control plots with size of 12m×24m i) fipronil G 0.2% 15 kg/ha (Positive control) and ii) were untreated (Negative control) with three replications of each were also included in the trial. Each trap was baited with the above-mentioned doses and replaced at 4-weeks intervals. Two sprays of insecticides were done in the control plot after 3 weeks intervals. Based on first-year results, a median dose of 250µg/20µL per rubber septum, with the binary mixture (50:50) were tested at 4.5 feet height of the trap with the trap densities at 8, 12, 18 and 25 traps/ha with three replications (Fig. 1).

Sex pheromone doses and trap heights: During the first year of study, the blend ratio (50:50) of two sex pheromone components (Z)-11-hexadecenal and (Z)-9-hexadecenal were used at different concentrations such as 50, 100, 200, 300 and 500µg/20µL in rubber spectrum with each trap in the rice field, synthetic pheromones and commercial pheromone trap devices were bought from Zafar Shani Chemical Company, Multan, Pakistan.

Evaluation of treatment effects & crop damage assessment: Impact of treatments was determined by capturing the population of *C. suppressalis* in the pheromone trap and to evaluate the crop damage assessment by inspecting the two major types of symptoms (white ears and dead hearts) were observed. Rice crop damage symptoms were assessed by counting the infested plants of rice field by examining 200 plants randomly selected from each application including control plots. Stems and tillers of plant with symptoms were confirmed that the damage was caused by *C. suppressalis*. Damage assessments were carried out at beginning of September-November for rice growing kharif season (Fig. 1). The percentage of dead heart and white ears were calculated by using the previously standard formulas of Onate (1965) and Abbott (1925), the following procedure was adopted:

$$DH\ or\ WE\ \% = \frac{Number\ of\ DH\ or\ WE}{Number\ or\ total\ tillers}\ X100$$

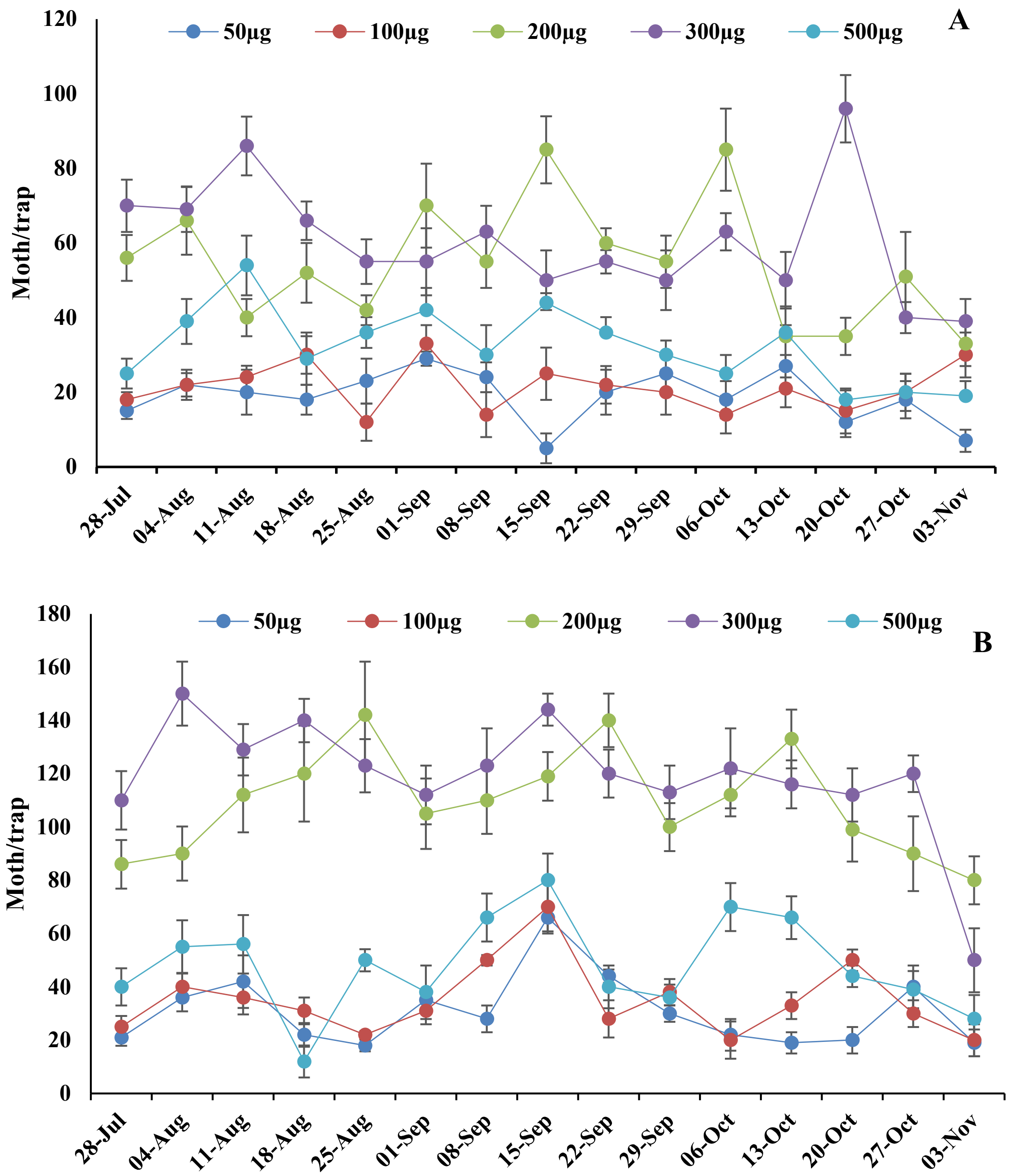
Whereas,DH= Dead heart in rice crop
WE= White ears of rice crop

Fig 1. Installation of sex pheromone trap (A), male moth captured in the trap (B), damage assessment (C &D).

Statistical analysis: Average data values for adults caught from all treatments and crop damage symptoms percentage and grain yield per plot were analyzed through one-way analysis of variance (ANOVA) followed by the LSD test to determine treatment differences. Efficiency was considered significantly different at (P ≤ 0.05), data analysis was carried out by using SPSS version 21 and graphs were generated with the assistance of Microsoft excel.

RESULTS

Efficacy of different sex pheromone dosage: During the first-year trial, weekly inclination trend of *C. suppressalis* (Fig. 2) and overall seasonal population trend (Fig. 1) were recorded from pheromone traps in the rice field, by testing different doses of two sex pheromones Z11-16: Ald and Z9-16: Ald (binary mixture) at 200 and 300µg/20µl per rubber septum with the same height positions caught significantly more (116.58±9.15/trap) and (101±8.17/trap) moths of *C. suppressalis* respectively as compared to the other doses (50, 100 and 500µg/20µl per rubber septum), and significant difference was observed between all pheromone dosage (df=15, F= 52.36, P ≤ 0.027).



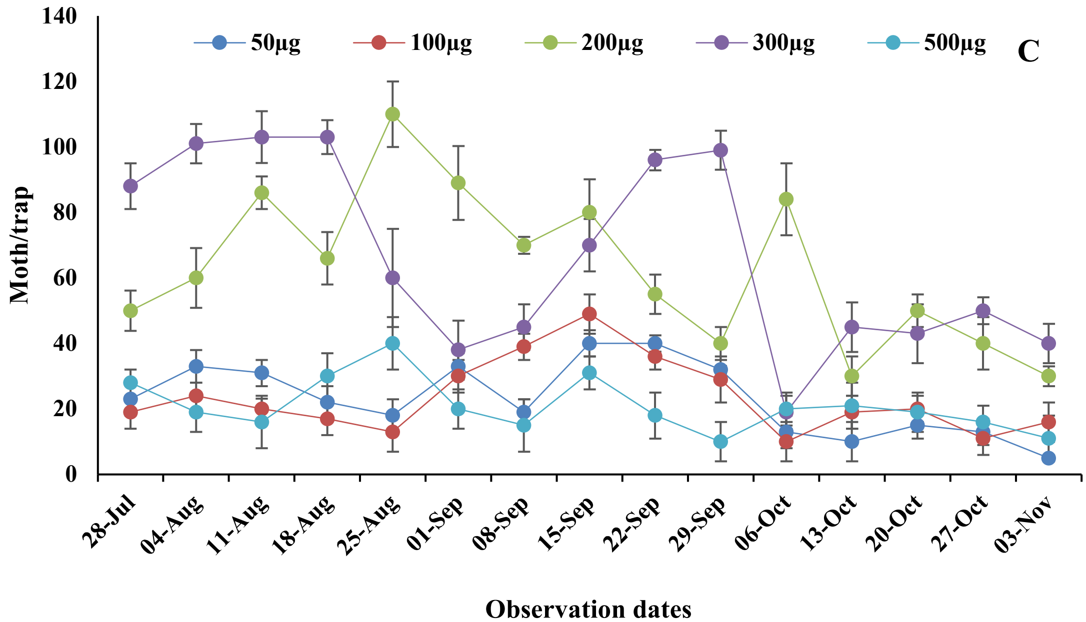


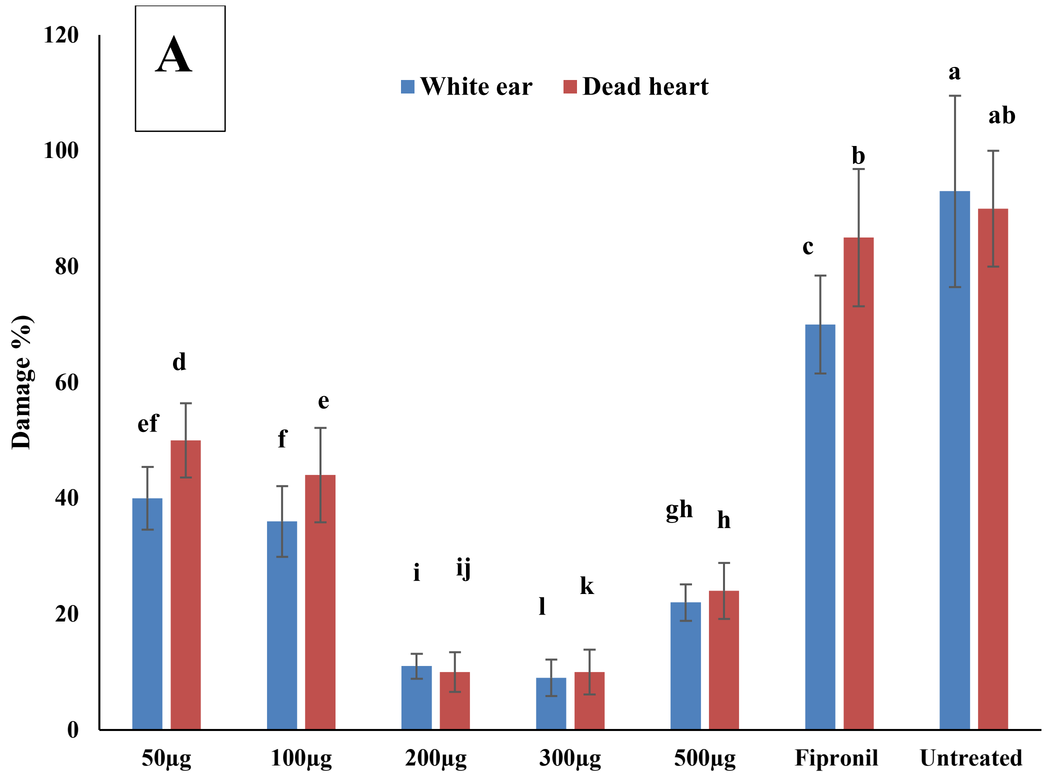
Fig. 2. The average weekly response of the captured population of *C. suppressalis* at different pheromone concentrations at different trap heights during 2021. A: Pheromone trap installed at 3 feet, B: Pheromone trap installed at 4.5 feet and C: Pheromone trap installed at 6 feet height.

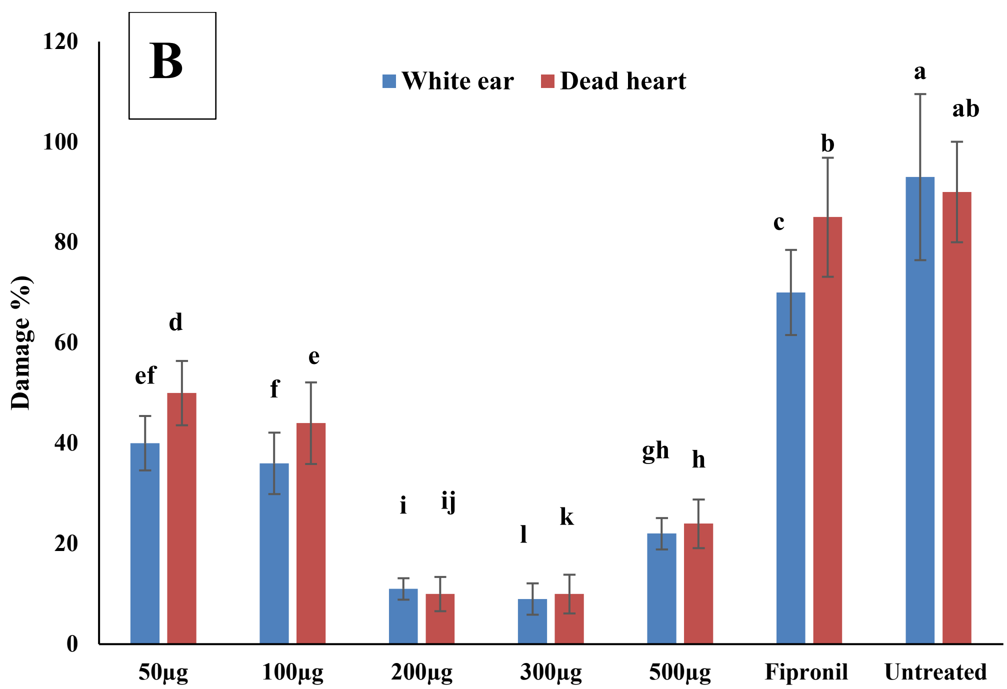
Table 1. Olfactory response of *C. suppressalis* towards the different pheromone concentrations and trap heights during 2021. Different letters indicate the difference between the concentrations of each trap position.

Trap heights (Feets)	Sex pheramone concentrations				
	50µg	100µg	200µg	300µg	500µg
3	18.86±2.15 j	21.33±2.89 hi	54.66±8.87 e	34.46±4.09 de	32.2±5.87 g
4.5	30.8±4.15 gh	34.93±5.74 g	109.2±20.14 b	121.93±19.77 a	48.01±6.58 f
6	23.13±3.48 h	22.8±4.25 i	62.66±7.21 d	66.12±6.54 c	20.9±3.80 hi

Evaluation of trap placement height: Different trap heights significantly captured *C. suppressalis* male moths (Table 1), traps placed at 4.5 feet captured maximum (121±19.77/trap) moths followed by 6 feet height (66.12±10.06/trap) moths. Whereas traps installed at 3 feet height were found less effective as compared to other heights and captured minimum (34.46±4.09/trap) moth. Statistical analysis showed a significant difference between all height treatments (df=9, F= 18.5, P≤0.001).

Crop damage assessment. Data from this trial demonstrated a distinctly significant difference (df; 03, F=9.90, P ≤ 0.0005) in both crop infestation symptoms (white ear and dead hearts) among all tested pheromone dosage. The minimum crop damage symptoms of white ears (07.86±1.89%) were recorded when pheromone traps installed at 4.5 feet with the dosage of 250µg followed by 300µg (10.43±2.06%) and 500µg (13.66±2.10%) were recorded respectively. Similarly, minimum crop damage symptoms of dead hearts (06.36±1.32%) were recorded when pheromone traps installed at 4.5 feet height with the dosage of 250µg, 300µg (08.93±1.50%), 500µg (11.95±1.90%) respectively. However, significant difference was observed dead hearts crop damage symptoms between the dosage (df; 03, F= 8.16, P≤0.002). In addition, control plots showed highest damage symptoms, white ear (85.25±20.03%) and dead hearts (90.52±18.03%) (Fig. 3).


A
White ear
Dead heart
Damage %)
0
20
40
60
80
100
120
ef
d
f
e
i
ij
l
k
gh
h
c
b
a
ab
50µg
100µg
200µg
300µg
500µg
Fipronil
Untreated


B
White ear
Dead heart
Damage %)
0
20
40
60
80
100
120
ef
d
f
e
i
ij
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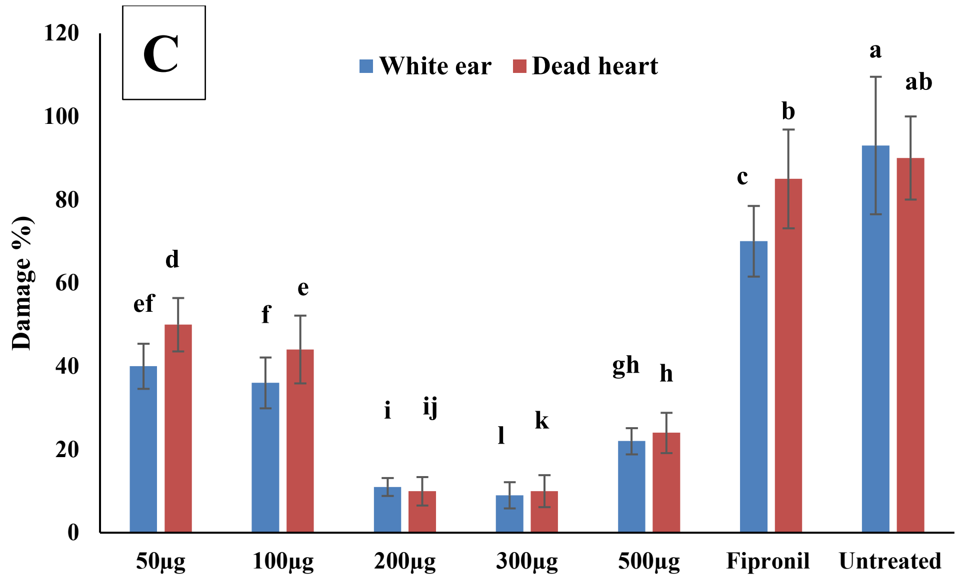

Fig. 3. Crop damage assessment caused by *C. suppressalis* after utilizing the different pheromone concentrations and various height during 2021. A: Installation of pheromone trap at 3 feet, B: 4.5 feet and C: 6 feet height in the rice field.

Based on the findings of first-year trials, a median average dosage of 250µg/20µl per rubber septum in each trap was further used to examine the most optimal trap density for the mating disruption of *C. suppressalis* with a unique trap height (4.5 feet) (Fig.4). Consequently, remarkable difference was found in different trap densities (df=3, F=15, P≤0.0039), the maximum mean (130.50±8.67/trap) moths were caught by 18 trap/ha, as compared to 25traps/ha (61.34±7.45), 12 traps/ha (50.87±6.54/trap) and 8 traps/ha (48.00±6.45/trap) respectively (Fig. 5).

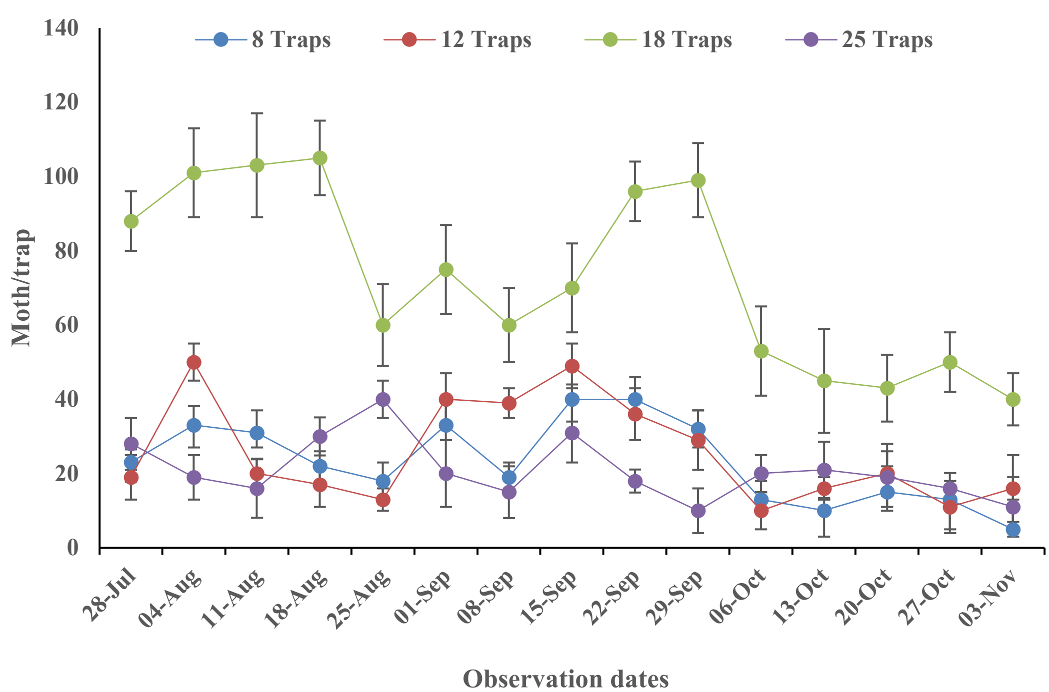

Fig. 4. Response of weekly captured population of *C. suppressalis* towards the pheromone trap densities during 2022 at effective pheromone dosage (250µg/20µl).

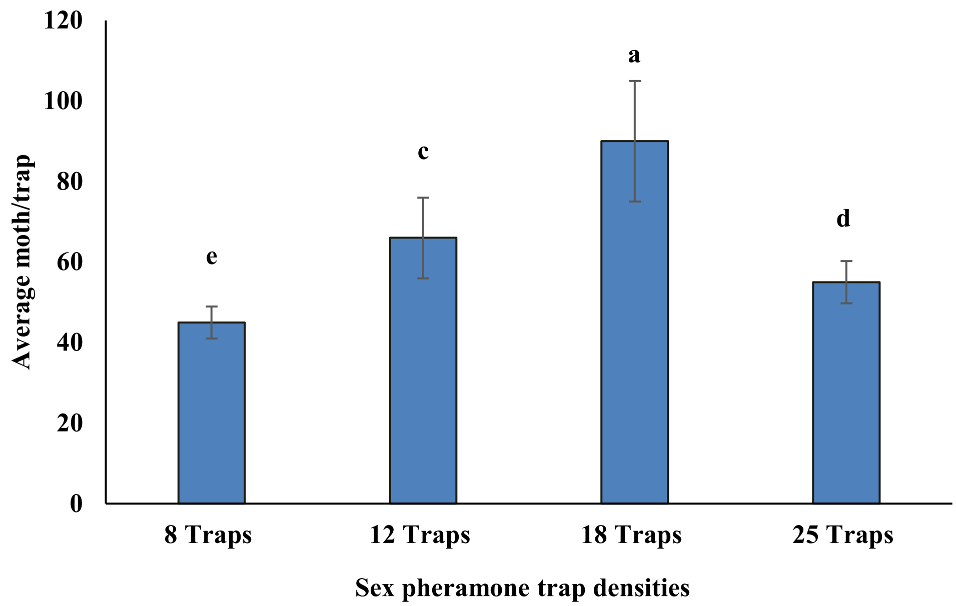

Fig. 5. Olfactory response of *C. suppressalis* towards the various densities of pheromone trap at 250µg/20µl during 2022.

Impact of sex pheromone trap densities on the population *C. suppressalis:* During the second year (2022) of research, a significant difference was observed between various trap densities including insecticide and control plots (Fig. 5). There was significant incidence differences noted in the symptoms of white ears (df=5, F= 41.1, P≤0.001) and dead hearts (df=5, F= 22.0, P≤0.031) in the different trap densities and other treatments. During next season experiments, less (10.02±2.37 %) damage symptoms were found where 18 sex pheromone traps/ha were installed, control and insecticide plots showed maximum damage symptoms with significant differences (P ≤0.001).

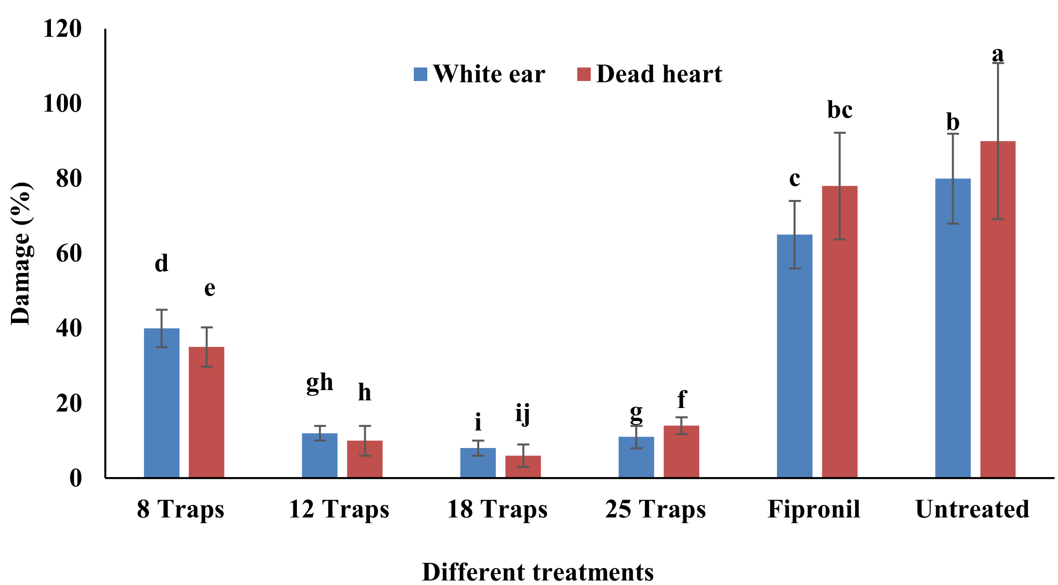

Fig. 6. Crop damage assessment caused by *C. suppressalis* after utilizing the different trap densities installed at 4.5 feet during year 2022.

Cost benefit ratio calculated for management of *Chilo suppressalis*: Impact of appropriate sex pheromone dosage, trap placement height and trap density on yield difference, net income, and cost/benefit ratio were compared with other treatments & control plots. The table 2 reveals that minimum grass expenditure/ cost to be incurred on the applications of 18 pheromone traps/ha was 7.90$ with highest (7400 kg/ha) yield, whereas maximum 18.76$ expenditure with lower (3917 kg/ha) rice yield recorded with the application of insecticide, also lowest (2801kg/ha) yield was recorded at control plots.

Table 2. Cost-benefit ratio of treatments.

Treatments	**Dose/ha**	**Yield kg/ha**	**Common cost/ha** ($)	**Cost of treatment /ha** ($)	**Total cost** ($)	**Gross income/ha** ($)	**Net income /ha** ($)	**Cost: benefit ratio**
Pheromone traps	18 traps	7400	592.51	7.90	601.44	2662.87	2080.52	1:3.52
Fipronil G 0.2%	15 kg/ha	3917	592.51	18.76	613.59	1408.99	796.50	1:2.30
Control (untreated)	-	2801	592.51	--	592.51	1007.05	401.12	1:0.06

DISCUSSION

Olfactory communication between the conspecific mates is usually mediated by sex pheromone and since the last three decades, sex pheromones are widely used for mating disruption in moths, but optimal doses and traps density is still unclear for maximum mating disruption. *C. suppressalis* is a noxious insect pest of rice which causes massive losses in every season (Chi *et al*., 2005; Litsinger *et al*., 2009). Mainly pest control through insecticides, which are not efficient due to cryptic feeding habits as well as have a serious impact on environment (Graham and Peter, 2009; Huang *et al*., 2003), thus, the pest control should be based on seized the reproduction through mating disruption technique. This does not only provide the protection of the crops by controlling the moths but is considered a possible alternative of synthetic chemicals and as an eco-friendly technique (Chen and Klein, 2012). The use of pheromone traps for mating disruption of *C. suppressalis* are expensive for the farming community since this technology is still not fully commercialized, due to lack of dosage optimization & appropriate height information. The optimal dose of sex pheromone could exhibit the efficient mating disruption in moth species. Recently, use of sex pheromone mating disruption techniques has become one of the most important and eco-friendly techniques to control lepidopteran pests on rice (Cui and Zhu 2016; Alfaro *et al*., 2009). During current research, initially different doses were used to determine the optimal dosage required for successful mating disruption through analysis of various dosage applications at different trap heights to control *C. suppressalis* in rice fields. Several studies reported the influence of trap design dosage and placement height on the capture size of male moths (Nwanze *et al*., 2021; James *et al*., 2011). Another important parameter to enhance trap catch is determining the optimal placement height of traps (Malo *et al*., 2004). Usually, insect flight plays a significant role in development and reproduction, suitable height of trap will capture the maximum flying insects as previously Pathan *et al*., (2023) conducted experiment on trap different height (30, 60,90 & 120 centimeter), and achieved remarkable results by capturing maximum thrips population on 90-centimeter heights. Also, Abbasi *et al*., 2008 installed sticky traps on different heights to capture the population of Asian ambrosia beetle on mango orchards & find out the appropriate trap placement heights, these previous studies confer that there are linkages between insect flight and appropriate trap height placement. Our result revealed that there was a direct association between the number of borer male moth captures and trap placement height, maximum moth was captured when sex pheromone traps deployed at 4.5 feet height above the ground. Recent studies (Witzgall *et al*., 2008; Stelinski *et al*., 2008) used different pheromone dispensers with different densities and dosage to monitor and control *Phyllocnistis citrella* (Su *et al*., 2003) as well as *C. suppressalis* (Alfaro *et al*., 2009; Wang *et al*., 2011). The perception mechanism of olfactory system in moth species is directly associated with the detection of pheromone plumes at different concentrations (Kaissling, 2001). Alfaro *et al*., 2009 tested different pheromone traps densities 16, 25 and 31 traps/ha, also 400 and 500 pheromone dispensers were used by Chen *et al*., (2014). Optimum or moderate dosage found more effective as compared to minimum of maximum dosage or trap densities, during current research, moderate doses such as 200 and 300µg/20µl per trap were proved the most optimum doses for mating disruption against this (rice stem borer) noxious insect pest. In the second year of study, average dose of 250 µg/20µl per rubber spectrum in each trap was utilized with different trap densities 8, 12, 18 and 25 trap/ha, where 18 traps/ha was proved the obvious difference as compared to the other trap densities in terms of average moth caught and damage symptoms. Targeting to the mating disruption management of *C. suppressalis* by using pheromone traps; assessment of the crop damage can directly provide the clues related to the treatment potential. The frequency of damage symptoms indicates that applications of pheromones could significantly reduce both white ears and dead hearts caused by *C. suppressalis* more efficiently than controls (Fipronil 0.4G and untreated). Our results are close agreement with Hossein *et al*., 2019; Souren and Roy (2022) they tested the sex pheromone management effects on *C. suppressalis* and *C. medinalis* and reduce 40-60% damage reduction in rice growing areas, respectively.

Conclusion: It may be concluded that damage symptoms were reduced progressively after installation an optimum dose of 250µg/trap and 18 traps/ha at the height of 4.5 feet. These are suitable for the management of rice stem borer that can achieve better results for moth arresting in rice-growing areas and consequently minimizing the costs of application and increase the yield of rice. Thus, the present research not only provides the essential clues to adopt the mating disruption technology for managing the rice striped stem borer is more beneficial as compared to the synthetic insecticides which to control lepidopteron moths to increase rice production for the betterment of farming communities.

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