Pheromone Characterization and Behavioral Responses of the Local Rice Yellow Stem Borer (*Scirpophaga incertulas*) to Different Sri Lankan Rice Varieties

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Abstract

The Rice Yellow Stem Borer (YSB), Scirpophaga incertulas (Lepidoptera: Crambidae), is a major pest that poses significant yield losses in Sri Lankan paddy cultivation. Synthetic chemical insecticides are the primary control method. However, their long-term effectiveness is limited due to negative impacts on biodiversity and risk to human health. This study aims to characterize the pheromone components of the local YSB population and assess their behavioral responses to different Sri Lankan rice varieties. Pheromone components were collected from female YSB using dynamic headspace collection (30 individuals per replicate) and solvent extraction (15 individuals per replicate). A varietal preference test was conducted using a dual-choice olfactometer for eight rice varieties: BG 366, AT 311, BG 257, AT 362, BG 251, AT 313, BG 360 and BG 377. The collected volatile compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis identified (Z)-11-Hexadecenal as a major sex pheromone compound in YSB. The varietal preference test shows significant differences (p = 0.001) among the rice varieties. Notably, AT 313 is the most attractive rice variety (13.2 ± 0.33), and BG 251 is the least attractive rice variety (3.8 ± 0.22) to the female YSB. S. incertulas most prefers to damage paddy cultivation after the pre-booting stage (early panicle initiation). The optimum laboratory rearing conditions for S. incertulas were determined as 26 ± 2 ^{0}C temperature, 75 ± 5% relative humidity, and a 10-12 hours light: 12-14 hours dark cycle. Further studies are necessary regarding laboratory bioassays and field evaluations to develop a sustainable and effective semiochemical-based pest management strategy (pheromone trap) to manage the rice yellow stem borer more effectively.

Keywords: Dynamic headspace collection, GC-MS analysis, Pheromone gland extraction, Rice yellow stem borer, Sustainable pest management

I. INTRODUCTION

Rice (*Oryza sativa*) is the most widely produced cereal crop, serving as a staple diet for more than half of the global population (Dhruv *et al*., 2019). Its cultivation is essential for food security, especially in Asian regions, including Sri Lanka. In Sri Lanka, paddy is cultivated on approximately 708,000 hectares of land and yields 5.037 million metric tons annually (Department of Census and Statistics, 2023). However, paddy cultivation is highly vulnerable to various pests that cause significant yield losses.

However, paddy cultivation is highly vulnerable to various pests. Among over 100 pest species affect paddy cultivation, only a few causes damage beyond the economic threshold level (ETL) (Muralidharan & Pasalu, 2006). These species are known as major pests (Sai *et al*., 2022), including the brown plant hopper, gall midge, rice leaf folder, and rice yellow stem borer. The rice yellow stem borer (*S. incertulas*) is one of the most destructive pests (Raut *et al*., 2017), causing yield losses of 10-60% (Sah & Sharma, 2023). Larvae are bored into the stem of paddy plants while disrupting the vascular system (Arun Kumar & Devanand R. Bankar, 2023). It inhibits the nutrient flow and reduces plant growth and yield. "Dead-hearts" are affected during the vegetative growth stage, and "Whiteheads" (Anis Syahirah Mokhtar *et al*., 2024) are affected during the reproductive stage.

Therefore, sustainable pest management strategies are essential to ensure food security and protect the economic stability of paddy farmers. Existing cultural, biological, mechanical, and

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chemical control methods are ineffective due to the environmental factors and variation of *S. incertulas* population dynamics. An eco-friendly and effective pest management strategy is essential to control the *S. incertulas* population according to local conditions (Batta *et al*., 2023). However, no significant attempts have been made to utilize semiochemical and plant volatiles analysis for its control. This study aims to was to identify and characterize YSB female sex pheromones and attractant and repellent varieties for management of YSB in paddy cultivation.

II. MATERIALS AND METHOD

A. Experimental Site

The research was conducted from October 2024 to April 2025 at the Crop Science and Technology Laboratory in the Faculty of Technology, South Eastern University of Sri Lanka.

B. Sample Collection and Rearing

Live *S. incertulas* larvae were collected from paddy fields exhibiting signs of stem borer infestation. Sample collection was done in the 2024 Maha season at the Rice Research Station, Sammanthurai. Collected larvae were brought and reared under particularly laboratory conditions, 26 ± 2 ^{0}C temperature, 75 ± 5% relative humidity, and a 10-12 hours light: 12-14 hours dark cycle. Potted healthy paddy cultivation was maintained prior to initiating the rearing procedure. The larvae of *S. incertulas* were provided with paddy plants early panicle initiation stage as a food source and maintained until pupation and adult emergence. Newly emerged adults were transferred to cages and provided with a 10% sugar solution-soaked cotton pads as their food source. Paddy plants (vegetative stage) were introduced into cages as oviposition substrates. Egg masses were collected and new larvae emerging from the eggs follow the same procedure.

C. Gender Identification

The gender identification of moths was carried out based on morphological characteristics using both macroscopical and microscopical characters. Female moths were larger than males, who are also bright yellow and have black spots in the middle of their forewings, with a wide abdomen. Male moths were light brown and had rows of black spots on the ends of the forewings, with a slender abdomen. Female moths that produce sex pheromones to attract male moths were selected for the extraction of pheromone compounds.

Figure 01: Gender Identification of *S. incertulas*

D. Extraction of Pheromone Compounds

Two methods were used to extract the pheromone compounds from female YSB. It ensures a thorough and accurate analysis of the pheromone profile.

The solvent extraction of pheromonal compounds was done at during the peak pheromone-releasing period (calling period between 23:00-03:00 h). The pheromone glands of virgin female moths (15 moths per replicate) were isolated using a micro-insect dissecting tool. The glands were immediately dissolved with 0.5 ml HPLC dichloromethane (DCM). The solvent containing the extracted pheromones was filtered using microfiltration and concentrated with ultra-pure nitrogen (N_2) gas and stored at -20 ^{0}C until GC-MS analysis.

The dynamic Headspace Collection method was used to collect pheromones from live virgin females during their peak calling period. A dynamic headspace sampling apparatus was used for the study. The setup contained an air-tight glass container connected to a charcoal filter at one arm, which ensured the purity of the air circulating through the air inlet, and Porapak Q adsorbent trapped volatile compounds of the other arm. A suction pump was used to draw air through the Porapak Q adsorbent tube. Female moths (30 moths per replicate) were placed in the air-tight glass container, and the pheromone released during their calling period was extracted. The trapped pheromonal compounds in the Porapak Q absorbent were removed after 24 h

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into a GC-MS vial using 0.5 ml of DCM (HPLC group). It was stored at -20 ^{0}C until analysis.

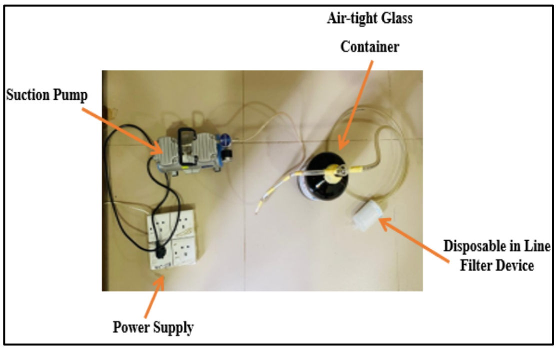

Figure 02: Dynamic Headspace Setup

E. GCMS Analysis

The extracted pheromone samples were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to identify volatile pheromone compounds. GC-MS consisted with GC 8890 coupled with a 5977B MSD, Agilent Technologies. The analysis was performed using a capillary column (HP5MS, 30 m x 0.250 mm x 0.25 µm) with helium (99.99%) as the carrier gas at a flow rate of 1.2 mL/ min. A 2 µl sample was injected into the system. The oven and column temperature were initially set at 35 ^{0}C for 1 minute and then ramped up at a rate of 10 ^{0}C/min to 230 ^{0}C. The separated constituents were identified by comparing mass spectra to the National Institute of Standards and Technology (NIST) library.

F. Varietal Preference Test

This study was conducted to evaluate the varietal preferences of adult female *S. incertulas* using a dual-choice experimental series. A dual-choice olfactometer was used for the experiment. The setup was designed to distribute environmental factors and other internal factors equally to avoid bias in the preference.

The paddy varieties, namely, BG 366, AT 311, BG 257, AT 362, BG 251, AT 313, BG 360, and BG 377 were grown as seedlings. Two-week-old paddy seedlings were placed at the olfactometer to ensure equal exposure.

For this purpose, two-week-old paddy seedlings of each variety were placed on either side of an experimental setup. Twenty adult female *S. incertulas* were released into the central compartment of the setup and allowed to move freely between the two rice varieties. The number of females present on each rice variety was observed and recorded separately in the morning (9:00 h) and evening (16:00 h).

Figure 03: Varietal Preferences Test Setup

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test at a 5% significance level using the SPSS software package (version 25).

The dynamic Headspace Collection method was used to separately collect volatile compounds emitted from both the most preferred and the least preferred paddy varieties. The samples were analyzed using GC-MS to identify volatile components. This information suits pest management strategies based on varietal resistance.

III. RESULTS

A. Pheromone Identification Using the Pheromone Gland Extraction Method

Gas chromatography-mass spectrometry (GC-MS) analysis of pheromone gland extracts discovered pheromones and volatile organic components. Two pheromone compounds were identified from *S. incertulas* females in Sri Lanka. (Z)-9-Hexadecenal, which was recorded at 5.731 min of retention time, and (Z)-11-Hexadecenal, which was recorded at 11.355 min of retention time, were identified as major pheromone compounds.

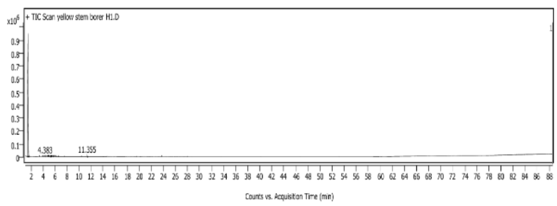

Figure 04: GC-MS Chromatograph of volatile organic compounds extracted from the solvent extraction method of *S. incertulas* females

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Other volatile organic compounds were included: Benzene, Toluene, Phenol, and Hexanol, with different retention times. These were known as metabolic byproducts, host plant volatiles, and semiochemicals influencing feeding/ oviposition.

Table 01: Volatile organic compounds extracted from the solvent extraction method of *S. incertulas* females

Retention Time	Name of the Compound	Putative Biological Role
4.383	Benzene	Metabolic byproducts
5.248	Toluene	Host plant volatile
5.288	Phenol	Semiochemical influencing feeding/ oviposition
5.401	Hexanol	Host plant volatile
5.445	Pinene	Host plant attractant or repellent
5.731	(Z)-9-Hexadecenal	Minor sex pheromone component
11.355	(Z)-11-Hexadecenal	Major sex pheromone component

Note: The Table shows different volatile compounds of a blend of S. incertulas pheromone.

B. Pheromone Identification Using the Dynamic Headspace Collection Method

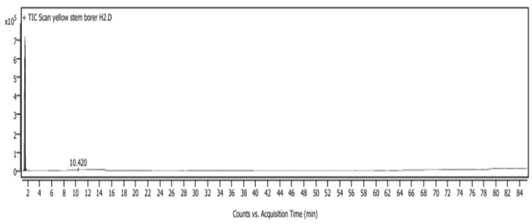

Figure 05: GC-MS Chromatograph of volatile organic compounds extracted from the dynamic headspace collection method of *S. incertulas* females

Two volatile organic compounds and one pheromone compound were identified. (Z)-11-Hexadecenal, recorded as a major female sex pheromone of *S. incertulas*, was recorded at 11.355 min of retention time. Benzene, recorded at 4.383 min of retention time, is considered a metabolic byproduct, and Hexanol, recorded at 5.401 min of retention time, is considered a host plant volatile.

Table 02: Volatile organic compounds extracted from the dynamic headspace collection method of *S. incertulas* females

Retention Time	Name of the Compound	Putative Biological Role
4.383	Benzene	Metabolic byproducts
5.401	Hexanol	Host plant volatile
11.355	(Z)-11-Hexadecenal	Major sex pheromone component

Note: The Table shows different volatile compounds of a blend of *S. incertulas* pheromone

C. Varietal Preferences of Rice Yellow Stem Borer

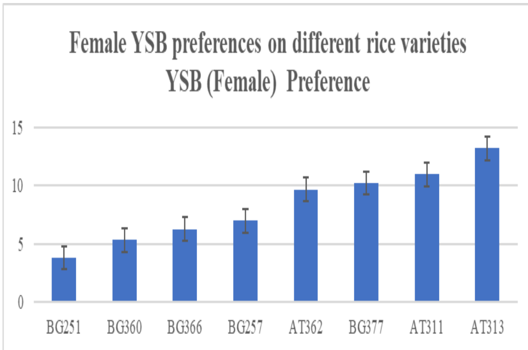

Figure 06: Female *S. incertulas* preferences on different rice varieties

According to the analyzed data, there were significant differences ($p = 0.001$) in Rice yellow stem borer preference among the 8 paddy varieties (BG 366, AT 311, BG 257, AT 362, BG 251, AT 313, BG 360, BG 377). The most preferred paddy variety was AT 313 (13.2 ± 0.33) to female yellow stem borer moths, and the least preferred variety was BG 251 to female yellow stem borer moths.

D. Volatile Identification of AT 313 paddy variety

Thirteen (13) Paddy-related volatile organic compounds (VOCs) were identified at different retention times in AT 313 paddy variety by GC-MS.

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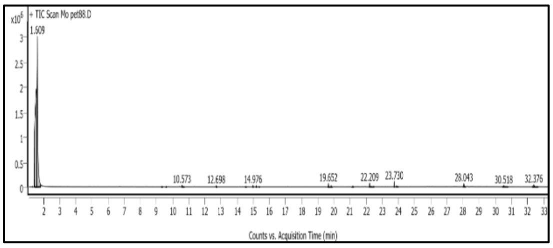

Figure 07: GC-MS Chromatograph of VOCs extracted from the dynamic headspace collection method of AT 313 paddy variety

Table 03: VOCs extracted from the dynamic headspace collection method of AT 313 paddy variety

Retention Time	Name of the Compound
10.573	1-Pentene, 4,4-dimethyl
12.704	1-Heptanol
15.192	Pentane, 2,2,3,4-tetramethyl
15.364	Oxalic acid, butyl propylester
19.824	1H-Tetrazol-5-amine
22.216	2,6-Bis(1,1-dimethylethyl)-4-methyl-4-isopropylcyclohexa-2
28.05	Acetic acid, trifluoro-,3,7-dimethyloctyl ester
32.382	Phthalic acid, cyclobutyl isobutyl ester
10.573	1-Pentanol, 2-ethyl-4-methyl
12.704	1-Hexanol, 4-methyl
28.043	1-Undecene, 9-methyl
9.6	Butane, 2,2-dimethyl
19.652	Cyclopropane, octyl-
10.573	1-Pentene, 4,4-dimethyl

Note: The table presents the major VOCs identified from the AT 313 paddy variety using the dynamic headspace collection method.

E. Volatile Identification of BG 251 paddy variety

A total of eighteen (18) VOCs detected by GC-MS at different retention times.

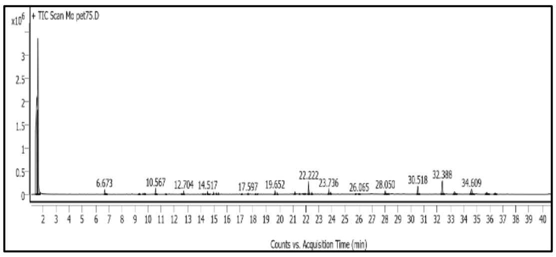

Figure 08: GC-MS Chromatograph of VOCs extracted from the dynamic headspace collection method of BG 251 paddy variety

Table 04: VOCs extracted from the dynamic headspace collection method of BG 251 paddy variety

Retention Time	Name of the Compound
17.171	Safrole
19.346	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-
19.505	Bicyclo [3.1.0] hex-2-ene,4-methyl-1-(1-methylethy
19.735	1-Hexanol, 4-methyl-
20.008	Methyleugenol
20.275	(Z, Z)-. alpha. -Farnesene
22.636	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-
23.526	3-Methoxy-4,5-methylenedioxybenzaldehyde
23.806	Cyclopentane, 1,1,3-trimethyl
24.315	Benzylphenethylamine, N-ethoxycarbonyl
14.995	Cyclopentane, 1,1-dimethyl
23.742	Acetic acid, trifluoro-,3,7-dimethyloctyl ester
10.579	1-Pentene, 4,4-dimethyl
12.704	Oxalic acid, butyl propylester
14.982	1-Heptene, 6-methyl
19.658	Cyclopropane, octyl
19.823	1H-Tetrazol-5-amine
28.05	Cyclopentane, (2-methylbutyl)
17.171	Safrole

Note: The table presents the major VOCs identified from the BG 251 paddy variety using the dynamic headspace collection method.

IV. DISCUSSION

The present study identified two major pheromone components in the Sri Lankan rice yellow stem borer *S. incertulas*. They are; (Z)-11-Hexadecenal as the major component and (Z)-9-Hexadecenal as a minor component. This finding is consistent with previous studies conducted in other countries in the world ((Kirsch, 1988);(Raut *et al*., 2017)). According to previous studies, (Z)-11-Hexadecenal was indicated as the major sex pheromone component relevant to the attraction of male *S. incertulas* (Cork *et al*., 1985). Minor pheromone compound often acts independently but act synergistically with major pheromone compounds to enhance male moth attraction. Combining major and minor pheromone compounds may provide a semiochemical-based control method with improved efficacy *(Witzgall et al*., 2010).

Other studies have shown the importance of host plant volatile compounds in influencing insect

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behavior (Verheggen *et al*., 2010), including oviposition and feeding (El-Ghany, 2019). Both the dynamic headspace collection method and the solvent extraction method were used to identify host plant volatiles such as phenol, benzene, and hexanol. Compounds such as hexanol were shown to attract *S. incertulas* females for oviposition and survival (Qian *et al*., 2024).

Laboratory-based dual-choice olfactometer-based varietal preference tests provided insight into *S. incertulas* preferences. According to the results of the varietal preference test, there were significant differences among the eight selected paddy varieties (BG 366, AT 311, BG 257, AT 362, BG 251, AT 313, BG 360, and BG 377). The most preference for AT 313 and the least preference for BG 251. GC-MS analysis of volatiles released by AT 313 revealed compounds such as 1-Heptanol and Pentane, which may increase its attractiveness to *S. incertulas* females (Xiong *et al*., 2024). BG 251 was characterized by high levels of Safrole and Methyleugenol. Those compounds may be insect repellent properties appeared to be less attractive. Some compounds may act as attractants or repellents for *S. incertulas* females. Compared to the volatile profile of AT 313, BG 251 showed differences in its emissions. Certain compounds were completely absent from BG 251, while others, which possibly associated with plant defense mechanisms, were more prominent.

These differences may account for the lower host preference observed in behavioral tests.

This will enable the establishment of an integrated management system based on volatile compounds in the varieties and pheromones of *S. incertulas* (Witzgall, 2001). Thereby reducing the dependence on chemical synthetic pesticides and contributing to the creation of a long-term semiochemical-based (Smart *et al*., 2014) eco-friendly management strategy for local population of *S. incertulas*.

V. CONCLUSION AND RECOMMENDATION

This study was focused on identifying and determining the efficacy of female sex pheromones of the YSB in Sri Lanka and the development of semiochemical-based management strategies to reduce dependence on chemical pesticides while minimizing the environmental and health impacts. According to the results, (Z)-11-Hexadecenal was detected as a major pheromone compound of the *S. incertulas* in both dynamic headspace collection and pheromone gland extraction methods. (Z)-9-Hexadecenal was detected as a minor pheromone compound of the *S. incertulas*. There were significant differences among the eight paddy varieties in the varietal preference test. Notably, AT 313 was the most preferred variety, while BG 251 was the least preferred. With the comparison of volatile organic compounds, differences were identified between BG 251, the least preferred paddy variety, and AT 313, the most preferred paddy variety of *S. incertulas*. There may be attractive and repellent compounds. Therefore, we need to do further studies of different compounds to identify their roles. Pheromone components, volatile components of the most preferred variety and the least preferred variety, and their differences should be confirmed. Further studies are necessary to formulate semiochemical-based attractants or repellents to control *S. incertulas* populations and confirm their effectiveness under field conditions.

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ABBREVIATIONS

- YSB : Yellow Stem Borer
- ETL : Economic Threshold Level
- N2 : Nitrogen Gas
- GC-MS : Gas Chromatography-Mass Spectrometry
- VOCs : Volatile Organic Compounds
- DCM : Dichloromethane
- HPLC : High-Performance Liquid Chromatography
- BG : Bathalagoda
- AT : Ambalantota
- ANOVA : Analysis of Variance
- MSD : Mass Selective Detector
- NIST : National Institute of Standards and Technology

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