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Evaluation of Volatile Compounds in Rice Bugs (*Leptocorisa acuta*) and Assessing their Behavioral Responses to Different Sri Lankan Rice Varieties

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Abstract -Rice bug (*Leptocorisa acuta*) is a major pest responsible for significant yield losses in rice. Although conventional chemical insecticides are effective, they pose environmental and health hazards, highlighting need for exploring alternatives such as semiochemicals and bio-insecticides. In Sri Lanka, no prior studies to identify the Volatile Organic Compounds (VOCs) associated with rice bugs. We aimed to characterize VOCs emitted by *L. acuta* and its behavioral responses over eight Sri Lankan rice varieties (BG 251, BG 257, BG 377, AT 313, BG 360, BG 366, AT 311, AT 362). VOCs were collected from male and female rice bugs (20 individuals per sex, 3 replicates) using dynamic headspace sampling and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). D-limonene identified as the dominant in females, while 2-octenal was in males. Behavioral assays using a four-choice olfactometer revealed varietal preferences: BG 377 was the most attractive (5.0 ± 0.89, 6.88 ± 2.33, 6.88 ± 1.67), while BG 360 was the least preferred (3.5 ± 0.33, 3.25 ± 0.78, 4.25 ± 0.73). However, statistical significance was only in one instance (p = 0.046), where others non-significant (p = 0.264, p = 0.552). These findings can be used for future development of semiochemical-based pest management.

***Keywords**:* Behavioral response, GC-MS, *Leptocorisa acuta*, Olfactometer, Rice varieties, Volatile Organic Compounds

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Introduction

(*Oryza sativa*) is one of the world's most important staple crops and is extensively cultivated in tropical and subtropical regions (Tsuboi, 2012). Originating in Asia, rice has adapted to a range of environments and continues to support millions globally (Dushani, 2009). Despite improved cultivation practices, the crop remains vulnerable to pest infestations and diseases that reduce yield and grain quality (Sharma, 2023)

One of the most damaging pests is the rice bug (*L. acuta*), which affects flowering rice by feeding on developing grains, causing sterility, shriveling, and poor market value (Gunawardena, 1993). Its life cycle and feeding behavior allow it to thrive across multiple Poaceae hosts like maize and sugarcane (Badhai, 2022). Yield losses in Sri Lanka from *L. acuta* infestations can range from 5% - 60%, especially during peak reproductive stages of the rice plant (Mandanayake, 2018).

Conventional pest control relies heavily on synthetic insecticides such as carbofuran and malathion (Tigga, 2018). However, this chemical dependency raises concerns over pesticide resistance, environmental degradation, and threats to non-target organisms (Dutta, 2016). Cultural methods like synchronized planting, mechanical removal, and biological controls using spiders and parasitoids have shown promise but remain underutilized or under-researched in large-scale settings (Kumari, 2016).

A growing body of research emphasizes the role of VOCs in mediating insect–plant interactions. Rice plants emit VOCs that can attract or repel insect pests, while pests themselves release semiochemicals used in mating and host selection (Gokila, 2024). Understanding the chemical ecology of L. *acuta* and Sri Lankan rice varieties could lead to the development of eco-friendly biopesticides and VOC-based monitoring or repellent systems.

This study aims to identify the VOCs emitted by male and female rice bugs, characterize their chemical profiles, and evaluate behavioral responses of rice bugs to VOCs released by various Sri Lankan rice varieties. The research contributes to the integrated pest management (IPM) toolbox by identifying natural cues that may reduce dependency on synthetic chemicals while supporting sustainable rice production.

Materials and Methods

Study Location

The study was conducted from November 2024 to March 2025 at the Faculty of Technology, South Eastern University of Sri Lanka, Oluvil (7.2970° N, 81.8500° E). The location characteristics of rice bugs where collected were sandy soils, a tropical climate with an average annual temperature of 29.32 °C, and an annual rainfall of approximately 1746 mm (climate, January 12, 2025).

Rearing of Rice Bugs under Controlled Conditions

Adult rice bugs (*L. acuta*) were collected from local paddy fields around the university premises and also Sammanthurai Rice Research institute and reared under controlled laboratory conditions. Rearing was conducted in transparent plastic cages maintained at temperature 25 – 30 °C, relative humidity between 70 – 80% and a 12:12 light and dark photoperiod conditions. Potted rice seedlings served as a continuous food supply. A separate rice nursery was maintained to ensure the consistent availability of fresh host plants.

Identification and Separation of Male and Female Rice Bugs

Adult rice bugs were separated into male and female groups prior to volatile extraction. Identification was conducted using both macroscopic and microscopic observations. Male and female *L. acuta* were identified and separated by first observing external body features, males appeared smaller and had narrow, tapered abdomens, while females were larger with broader, rounded abdomens and then confirmed under a stereo-microscope by examining the genital structures, where males showed a pointed aedeagus and females displayed a curved ovipositor used for egg-laying.

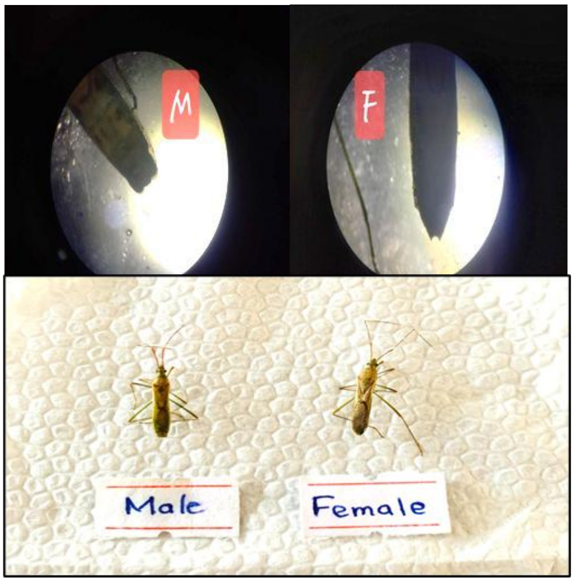

Figure 1: *Gender identification of paddy bugs*

Extraction of VOCs Separately from Male and Female Rice Bugs

For volatile compound extraction, male and female adult bugs were separated. Three replicates of 20 individuals were prepared for each sex. VOCs were collected using the dynamic headspace sampling technique. Insects were placed in airtight glass bottles fitted with Poropak-Q adsorbent tubes. Filtered air was pumped through the bottles, and emitted VOCs were trapped in the adsorbent material. The trapped compounds were eluted with dichloromethane (DCM) and stored at 4 °C for further analysis (GC-MS) (Peacock, 1975).

VOC Analysis via GC-MS

GC-MS was employed for VOC analysis. GC-MS analysis was conducted using an Agilent Technologies GC 8890 coupled with a 5977B Agilent Technologies MSD. The analysis was performed using a capillary column (HP5MS, 30 m× 0.250 mm × 0.25 µm) with helium (99.9%) 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 °C for 1 minute and then ramped up at a rate of 10 °C/min to 230 °C. The separated constituents were identified by comparing mass spectra to the National Institute of Standards and Technology (NIST) library (Garcia, 2011).

Behavioral Assays to Test VOC Attractant/Repellent Properties

Behavioral assays were performed using four-choice olfactometers designed to allow bugs to move freely between chambers containing different rice varieties. In the initial screening phase, all eight rice varieties (BG 251, BG 257, BG 377, AT 313, BG 360, BG 366, AT 311, AT 362) were tested in random combinations to observe insect preferences. Four replicates were conducted in both morning and evening sessions. The most and least preferred varieties were then re-tested in a confirmatory phase using two olfactometers, each with four varieties. Positions were rotated between replicates to eliminate directional bias (Saïd, 2006).

Data Collection and Statistical Analysis

Data collection involved counting the number of insects attracted to each variety and recording time taken to reach a choice. Statistical analysis was conducted using SPSS software (Version 25). One-way ANOVA was used to test significance in insect preference among varieties. Replication and randomization ensured robustness and reduced experimental bias.

Results

GC–MS analysis revealed clear differences in the volatile compound profiles of male and female *L. acuta*. The chromatogram of female rice bugs (Figure 2) showed a more complex and diverse volatile blend compared to males. Several major compounds were identified in females, including D-limonene, 2-octenal, citronellyl butyrate, and azulene, with D-limonene being the most abundant compound, as summarized in Table 1. In contrast, the chromatogram of male rice bugs (Figure 3) exhibited a simpler volatile profile. Male *L. acuta* primarily released 2-octenal and acetic acid octyl ester, with fewer detectable compounds than females.

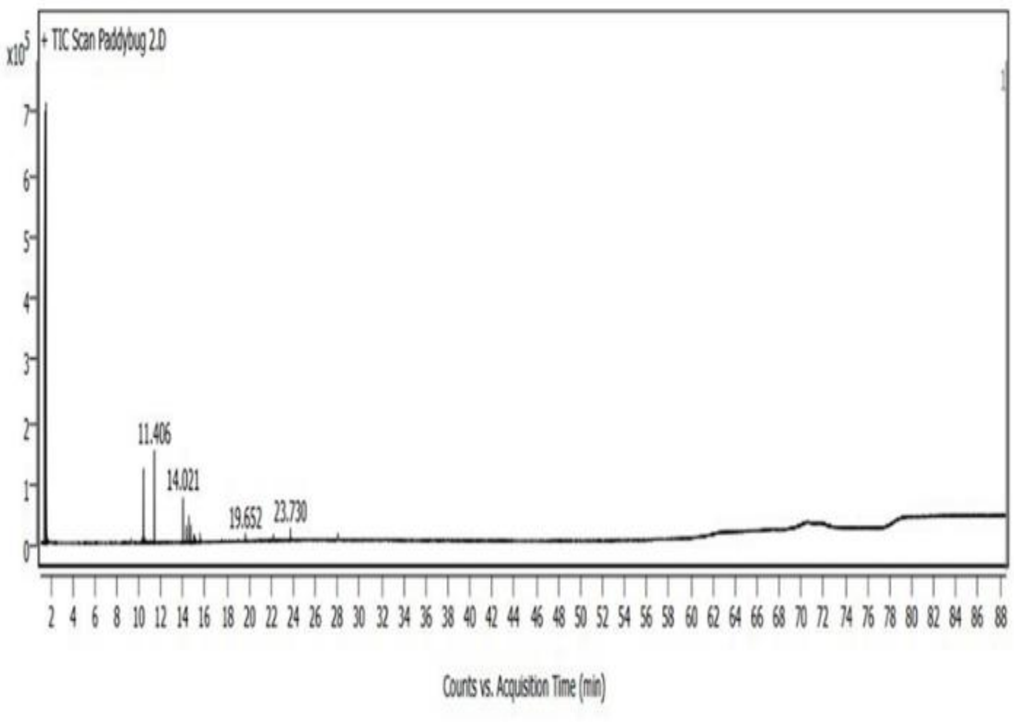

Figure 2: *GC–MS chromatogram of VOCs emitted by female L. acuta*

Table 1
Volatile compounds identified in female rice bugs

Compound	Retention Time (min.)
Methylene chloride	1.603
D-Limonene	10.433
2-Octenal	11.406
Cyclohexanone, 5-methyl-2	14.021
Unidentified	14.301
Citronellyl butyrate	14.518
Azulene	14.696
Unidentified	14.982
Unidentified	15.058
1-Hexanol, 4-methyl	15.542
Unidentified	19.652
Cyclopentane, trimethyl-	23.730

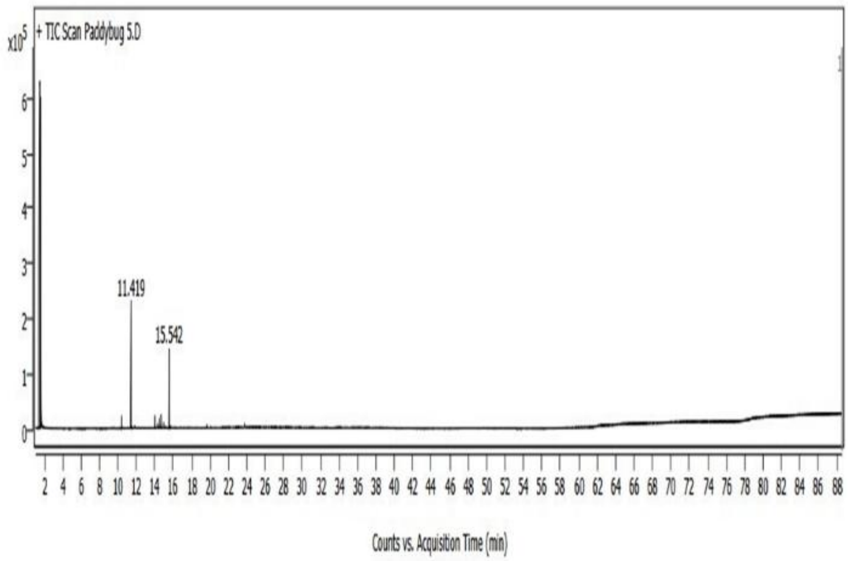

Figure 3: *GC–MS chromatogram of volatile compounds emitted by male L. acuta*

Table 2
Volatile compounds identified in male rice bugs

Compound	Retention Time (min.)
Methylene chloride	1.603
Unidentified	10.414
2-Octenal	11.419
Unidentified	14.002
Unidentified	14.505
Azulene	14.677
Acetic acid, octyl ester	15.542

Behavioral assays conducted using four-choice olfactometers demonstrated variability in rice bug attraction to eight Sri Lankan rice varieties. BG 377 showed the highest attraction, while BG 360 was the least preferred (Figure 4). Despite this observed trend, statistical analysis indicated no significant differences among the tested varieties ($p > 0.05$). This implies that although some rice varieties may numerically attract fewer rice bugs, their emitted VOCs may not differ strongly enough to elicit a consistent behavioral response. Factors such as minor differences in volatile profiles, environmental conditions, and insect physiological state may have influenced olfactory responses.

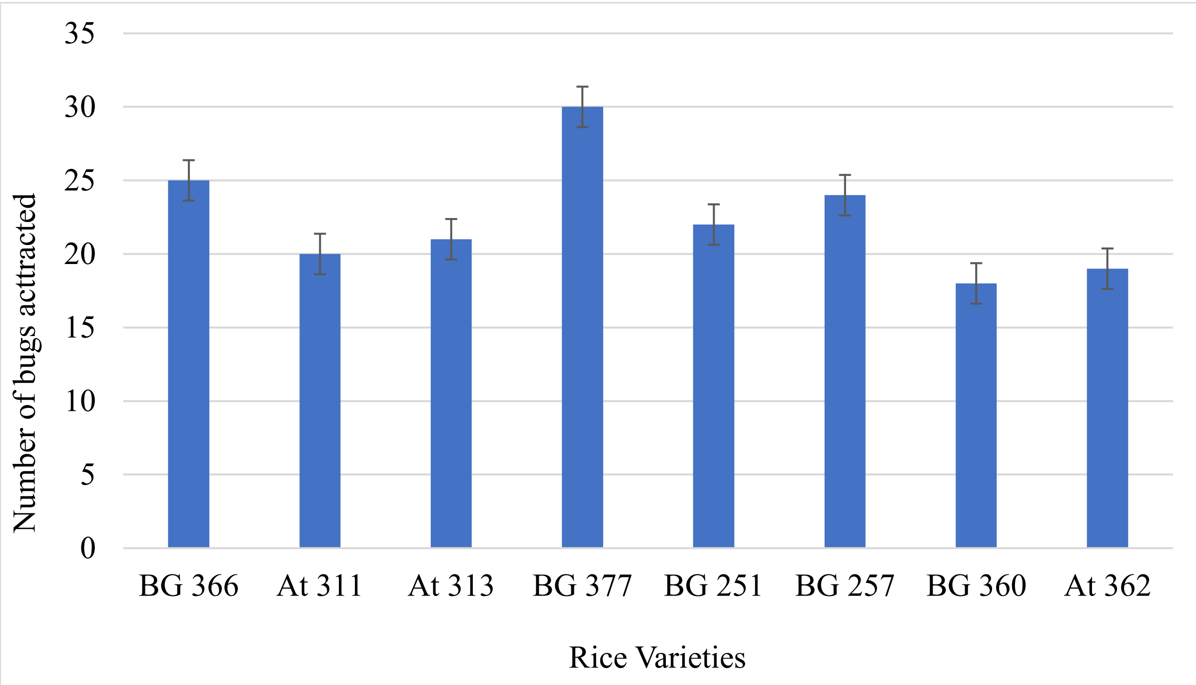

Figure 4: *Number of bugs attracted to the eight rice varieties*

Discussion

GC - MS analysis revealed clear sex specific differences in volatile emissions of *Leptocorisa acuta*, with females producing a more diverse blend of VOCs than males. This characteristic indicates their potential as a target for the development of semiochemical based pest management strategies.

The dominance of D-limonene and 2-octenal in females, and 2-octenal in males, suggests a potential role of these compounds in chemical communication. Similar aldehydes and terpenoid compounds have been reported in the closely related species *Leptocorisa chinensis*, where they function in aggregation and mating behavior (Leal et al., 1996). The detection of these compounds in *L. acuta* indicates conserved semiochemical traits within the genus *Leptocorisa*, although this study represents the first report of such compounds for *L. acuta* in Sri Lanka.

The simpler volatile profile observed in males compared to females is consistent with previous studies on *L. chinensis*, where females were identified as the primary emitters of pheromone-related compounds (Zhou et al., 2014). The presence of several unidentified compounds in both sexes suggests complex chemical signaling, where minor components may act synergistically to influence behavioral responses. Further electrophysiological and field-based studies are required to clarify the functional role of these compounds.

Behavioral assays showed variation in rice bug attraction among Sri Lankan rice varieties, with BG 377 being relatively more attractive and BG 360 less preferred, although most differences were not statistically significant. Similar weak but consistent varietal preference patterns have been reported for *Leptocorisa* species, where overlapping plant volatile profiles and environmental factors influence olfactory responses (Bruce et al., 2005). These findings suggest that rice bug host selection is mediated by complex VOC blends rather than single compounds, supporting the potential use of semiochemical-based strategies within integrated pest management programs.

Conclusion

The study explored the VOCs emitted by male and female rice bugs (*L. acuta*) and their behavioral responses to eight Sri Lankan rice varieties. GC-MS analysis revealed sex-specific VOC profiles, with females emitting D-limonene and 2-octenal, while males emitted simpler profiles dominated by 2-octenal. The presence of unidentified volatiles suggests complex chemical communication. Behavioral assays showed slight varietal preferences, with BG 377 being most attractive, but no significant differences were found between varieties. Overall, the findings enhance the understanding of *L. acuta*'s chemical ecology and provide a basis for developing semiochemical-based pest management strategies.

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