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RESEARCH ARTICLE

Unique volatile signature: herbivore-induced plant volatiles (HIPV) defense in three traditional Indian paddy varieties against brown plant hopper, *Nilaparvata lugens* (Stal.) infestation

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ABSTRACT

The southern Indian state of Tamil Nadu mainly cultivates traditional paddy varieties such as Chinnar, Karuppukavuni, and Karunguruvai that deploy a sophisticated defense system against herbivore attacks, emitting unique volatile signatures. Using gas chromatography-mass spectrometry, this study investigated these herbivore-induced plant volatiles produced in response to brown plant hopper infestation. Healthy plants emitted a limited range of compounds, while brown plant hopper damage triggered various HIPVs, including alcohols, esters, and iodine-containing molecules. Notably, herbivore-induced Chinnar displayed high levels of 2-butyl-2-ethyl-1,3-propanediol and 2-Nonen-1-ol, while Karunguruvai produced elevated 1,3-Propanediol, 2-butyl-2-ethyl. Specific ester profiles were also observed, with Dodecyl acrylate in Chinnar, 9-Octadecenoic acid (Z)-, phenylmethyl ester in Karuppukavuni, and Diethyl phthalate in Karunguruvai. Interestingly, both Chinnar and Karunguruvai emitted 1-Iodo-2-methylundecane upon brown plant hopper attack. These findings suggest that traditional paddy varieties employ a complex HIPV defense system. Further research on these volatiles could pave the way for eco-friendly pest management strategies in sustainable agriculture.

Keywords: *Brown plant hopper, HIPVs, Traditional rice varieties, Volatile organic compounds, Insect-Plant Interaction*

INTRODUCTION

Plants emit a sophisticated array of volatile organic compounds (Li *et al.,* 2019) from various organs, acting as chemical signals in their environment (Dudareva *et al.,* 2004). Interestingly, these plant volatiles are often produced in response to herbivore damage (Arimura *et al.,* 2005), influencing ecological interactions by attracting beneficial insects, deterring herbivores, and communicating with neighboring plants. Plants respond to herbivore feeding through increased biosynthesis and release of volatile substances from tissues that have been damaged (Paré and Tumlinson, 1999; Ruschioni, 2015). These volatiles often benefit the plant through the enhanced attraction of natural enemies (Dicke *et al.,* 1998; Turlings *et al.,* 1998;

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Kessler and Baldwin, 2001). Many plants release VOCs, which herbivores exploit as signals to select hosts (McCormick *et al.,* 2012).

Traditional paddy varieties like Karuppukavuni, Chinnar, and Karunguruvai, known for their historical and cultural importance in Tamil Nadu, have received different scientific attention than commercially grown rice. This study aims to address that gap by examining the volatile profiles of these three varieties under two conditions: healthy plants and those attacked by the brown plant hopper, *Nilaparvata lugens* (Stal.), a significant paddy pest.

Plants use volatile and non-volatile compounds to defend against herbivores (Yasmin *et al.,* 2021). These herbivore-induced plant volatiles include terpenes, green leafy volatiles, and various organic compounds like ethylene and methyl salicylate. Gokila *et al.* (2024) identified herbivore-induced volatile compounds in rice plants, viz., tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, heneicosane, tetracosane, hexacosane, octacosane, dotriacontane, tetrapentacontane, 2,6,10-trimethyldodecane, 2,6,11-trimethyldodecane, 11-methyltricosane, 1-nonadecene, 1-hexacosene, tetradecanoic acid, n-hexadecanoic acid and octadecanoic acid, from rice plants infested with insect herbivores, including yellow stem borer and leaf folder which likely attract natural enemies. Similarly, Shi *et al.* (2019) found that natural leaf rolling by a moth species triggered increased attraction of parasitic wasps in rice plants, suggesting the plants can perceive this behavior and emit volatile signals as a defense mechanism.

Zevallos *et al.* (2016) reported that the individual compounds emitted by maize upon feeding by the Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), elicit a response in conspecific females. Herbivory induced significantly the emission of b-myrcene, (Z)-3-hexenyl acetate, (E)-b-ocimene, b-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene [DMNT], indole, geranyl acetate, a-zingiberene, b-caryophyllene, (E)-a-bergamotene, (E)-b-farnesene, b-sesquiphellandrene, (E)-nerolidol, and 4,8,12-trimethyl-1,3,7,11-tridecatetraene. Kigathi *et al.* (2009) studied the emission of volatiles by *Trifolium pratense* (red clover) under laboratory and field conditions. They quantified 24 compounds, of which eight showed increased emission rates after herbivory by *Spodoptera littoralis* caterpillars. Most of those compounds have been reported as herbivore-induced volatiles from various plant taxa. de Assis *et al.* (2023) found similar hydrocarbon VOCs emitted by healthy and bacterial fruit blotch-infected watermelons using GC-MS.

MATERIALS AND METHODS

Identification of Herbivore-Induced Plant Volatiles (HIPVs) in rice ecosystem

To compare the variation in volatiles between the herbivore-infested and the healthy traditional varieties of paddy in Tamil Nadu, India. Seedlings were maintained to collect the volatiles from both samples. The seeds were procured from the Central farm, Agricultural College and Research Institute, Madurai. Seeds were sown in plastic trays, which were watered daily. After 14 days, the seedlings were transplanted to the mud pots. These pots were kept in an aluminum tray with water placed inside the iron-meshed screen house to prevent the invasion of insects. The collection of volatiles was carried out 25 days after transplanting.

Collection of volatiles using volatile collection unit

A volatile collection unit was used to collect headspace volatiles. The potted paddy plants were kept inside an air-tight glass jar. To prevent the emission of soil-related volatiles, the root portion of the plant was wrapped with aluminum foil, and brown plant hoppers were released at a rate of ten per plant. Headspace volatiles from the brown plant hopper-infested and healthy plants were collected into the adsorption tubes. Both sides of the adsorption tube were fitted with steel mesh grids to prevent the adsorbent from falling apart. Headspace volatiles were trapped for 2 hours, then the machine was stopped for half an hour, and collected for another 2 hours. The adsorbed volatiles were eluted immediately with 20 ml HPLC grade hexane. The eluted hexane extract was concentrated and injected into GC-MS for analysis (Reuolin *et al.*, 2022).

Identification of volatiles using GC-MS headspace unit

Headspace volatile compounds present in brown plant hopper-infested and healthy plants of traditional paddy varieties were identified using a Gas chromatograph coupled with mass spectrometry (GC-MS/MS) (GC 2010 plus, GCMS –TQ 8040 SHIMADZU) in the Department of Agricultural Entomology, Agricultural College and

Research Institute, Madurai. The gas chromatograph had a 30 m fused silica capillary column with 0.25 mm ID and 0.25 µm film thickness running in constant flow mode (1.40 ml/min helium). Oven temperature was programmed from 80 °C (1 min hold) to 100 °C at a 5 °C/min rate (1 min hold), then to 220 °C at a 10 °C/min rate (5 min hold), and then to 240°C at a 50°C/min rate (8 min hold). One microlitre of collected volatiles was loaded into glass vials with a capacity of 20 ml and kept in the headspace unit for analysis. Shimadzu GC-MS Lab solution software was used to analyze compounds in the volatiles. The compounds of interest were identified using the standard NIST 14 (National Institute of Standards and Technology) mass spectral library.

RESULTS AND DISCUSSION

The comparative analysis of volatile organic compounds (VOCs) emitted by healthy and brown planthopper (BPH)-infested plants of three traditional paddy varieties, namely Chinnar, Karuppukavuni, and Karunguruvai, revealed distinct differences in their volatile profiles. The variations in the composition and relative abundance of VOCs between healthy and herbivore-infested plants indicate differential chemical responses to herbivore attack among the three varieties. The findings highlight the diversity of volatile compounds associated with plant defence and suggest that these traditional rice varieties possess distinct chemical response patterns under herbivory.

Volatile profile of healthy and BPH-infested Chinnar

The volatile profile of healthy Chinnar plants revealed two compounds, namely 1,2,4-trimethylbenzene (aromatic hydrocarbon) and 2-ethyl-1-hexanol (alcohol), with peak areas of 1.24% and 1.51%, respectively (Table 1). In contrast, BPH-infested Chinnar exhibited a broader volatile profile, comprising dodecyl acrylate (ester), 2-butyl-2-ethyl-1,3-propanediol and 2-nonen-1-ol (alcohols), and 1-iodo-2-methylundecane (iodine-containing compound). Among these, 2-butyl-2-ethyl-1,3-propanediol recorded the highest peak area of 6.76%, followed by 1-iodo-2-methylundecane (5.47%) and 2-nonen-1-ol (2.43%). The appearance of additional compounds following BPH infestation indicates a change in the volatile composition of Chinnar in response to herbivore attack. In particular, the identification of 1-iodo-2-methylundecane, an iodine-containing compound, represents an interesting feature of the volatile profile of the infested plants, although its specific ecological function in rice defence remains to be established.

The differences observed between healthy and infested Chinnar plants can be interpreted in the context of herbivore-induced indirect plant defence. Plants release specific volatile blends following herbivore feeding, which may function as chemical signals that facilitate interactions with natural enemies, including parasitoids and predators (Degenhardt, 2009). Such herbivore-induced plant volatiles (HIPVs) can mediate interactions across trophic levels and influence the behaviour of organisms associated with the plant (Ali *et al.*, 2013; Dicke & Baldwin, 2010). The detection of additional alcohols and an iodine-containing compound in BPH-infested Chinnar suggest that herbivore attack is associated with changes in its chemical emission profile. Similar responses involving alcohols and other volatile compounds have been discussed in relation to plant defence against herbivores (Degenhardt, 2009). Nevertheless, the potential role of the individual compounds identified in Chinnar in attracting natural enemies or deterring herbivores requires further behavioural validation.

Table 1. Volatile profile of healthy and BPH-infested Chinnar, a traditional paddy variety

Name of the sample	RT	Name of the compound	Functional group	Peak area (%)
Healthy plants	5.69	1,2,4-Trimethylbenzene	Aromatic hydrocarbons	1.24
	6.53	2-Ethyl-1-hexanol	Alcohols	1.51
BPH-infested plants	31.36	Dodecyl acrylate	Esters	1.38
	33.43	2-Butyl-2-ethyl-1,3-propanediol	Alcohols	6.76
	36.58	2-Nonen-1-ol	Alcohols	2.43
	41.92	1-Iodo-2-methylundecane	Iodine-containing	5.47

Volatile profile of healthy and BPH-infested Karuppukavuni

Karuppukavuni exhibited a distinct change in its volatile profile following BPH infestation. In healthy plants, two ester compounds, namely phthalic acid, ethyl hept-3-yl ester and didodecyl phthalate, were detected, with peak areas of 1.32% and 2.54%, respectively (Table 2). Following herbivore infestation, the volatile profile comprised n-butyl nitrite, pentanoic acid, 3-methyl-, 9-octadecenoic acid (Z)-, phenylmethyl ester, 4-trifluoroacetoxytetradecane and 4,4,8-trimethyl-non-5-enal. Among these compounds, n-butyl nitrite recorded the highest peak area of 16.91%, followed by 4,4,8-trimethyl-non-5-enal (14.06%). The other identified compounds, including 4-trifluoroacetoxytetradecane, pentanoic acid, 3-methyl-, and 9-octadecenoic acid (Z)-, phenylmethyl ester, recorded peak areas of 3.41%, 2.87% and 1.42%, respectively. The appearance of a broader range of compounds, particularly the prominent occurrence of n-butyl nitrite and the aldehyde 4,4,8-trimethyl-non-5-enal, distinguishes the herbivore-induced volatile profile of Karuppukavuni from that of its healthy counterpart.

The volatile profile of BPH-infested Karuppukavuni further illustrates the diversity of chemical responses associated with herbivory. In particular, the relatively higher peak areas of n-butyl nitrite and 4,4,8-trimethyl-non-5-enal indicate their prominence in the detected volatile mixture. The occurrence of the aldehyde 4,4,8-trimethyl-non-5-enal is noteworthy, as aldehydes constitute an important group of plant volatile compounds associated with stress-related chemical signalling. Kutty and Mishra (2023) discussed the role of volatile information chemicals in inducing and regulating plant defence responses during herbivory. The findings therefore provide an additional perspective on the chemical diversity associated with herbivore-induced responses in rice. However, the specific contribution of the identified aldehyde and other compounds to herbivore deterrence or natural enemy attraction warrants further investigation.

Volatile profile of healthy and BPH-infested Karunguruvai

Among the three traditional paddy varieties, Karunguruvai exhibited the broadest range of detected compounds in its BPH-infested state. Healthy Karunguruvai plants showed the presence of a single compound, (+)-2-bornanone, a ketone, with a peak area of 1.47% (Table 3). In contrast, BPH-infested plants exhibited ten additional compounds belonging to different functional groups, including alcohols, esters, hydrocarbons, iodine-containing compounds and sulfur-containing compounds. These included 1,2,3,4,5-cyclopentanepentol, lactose, 1-iodo-2-methylundecane, diethyl phthalate, decane, 2,3,5,8-tetramethyl-, dodecane, 2,6,10-trimethyl-, 1,3-propanediol, 2-butyl-2-ethyl, 1,2-benzenedicarboxylic acid, butyl decyl ester and cyclic octaatomic sulfur.

Among the compounds detected in infested Karunguruvai, 1,3-propanediol, 2-butyl-2-ethyl recorded the highest peak area of 16.63%, followed by diethyl phthalate (6.74%) and cyclic octaatomic sulfur (3.60%). Dodecane, 2,6,10-trimethyl- and 1,2-benzenedicarboxylic acid, butyl decyl ester recorded peak areas of 3.34% and 2.14%, respectively, while decane, 2,3,5,8-tetramethyl- and

Table 2. Volatile profile of healthy and BPH-infested Karuppukavuni, a traditional paddy variety

Name of the sample	RT	Name of the compound	Functional group	Peak area (%)
Healthy plants	27.70	Phthalic acid, ethyl hept-3-yl ester	Esters	1.32
	38.32	Didodecyl phthalate	Esters	2.54
BPH-infested plants	7.48	n-Butyl nitrite	Diverse functional groups	16.91
	12.43	Pentanoic acid, 3-methyl-	Carboxylic acids	2.87
	43.13	9-Octadecenoic acid (Z)-, phenylmethyl ester	Esters	1.42
	43.45	4-Trifluoroacetoxytetradecane	Diverse functional groups	3.41
	44.28	4,4,8-Trimethyl-non-5-enal	Aldehydes	14.06

1-iodo-2-methylundecane accounted for 2.23% and 2.57%, respectively. The remaining compounds, 1,2,3,4,5-cyclopentanepentol and lactose, recorded peak areas of 1.06% and 0.76%, respectively. The presence of a diverse range of compounds, particularly the prominence of 1,3-propanediol, 2-butyl-2-ethyl, indicates a marked difference in the volatile composition of healthy and BPH-infested Karunguruvai. The detection of 1-iodo-2-methylundecane in both Chinnar and Karunguruvai is also noteworthy, suggesting that this compound was common to the herbivore-induced volatile profiles of these two traditional varieties.

The chemical profile of Karunguruvai further demonstrates the variation in volatile composition among the traditional paddy varieties following herbivore infestation. The prominence of alcohols and esters, together with the detection of hydrocarbons and sulfur-containing compounds, indicates a chemically diverse volatile mixture. The identification of 1-iodo-2-methylundecane in both Karunguruvai and Chinnar is particularly interesting, as it represents a shared compound in the herbivore-induced profiles of these varieties. Pinto-Zevallos *et al.* (2016) investigated herbivore-induced volatile organic compounds emitted by maize and their electrophysiological effects on female *Spodoptera frugiperda*, demonstrating the importance of examining the behavioural responses of insects to plant-derived volatile compounds. Similarly, the presence of diverse volatile compounds in Karunguruvai provides a basis for further investigations into their potential roles in plant–herbivore interactions.

Comparative interpretation of volatile profiles among the three traditional paddy varieties

A comparison of the volatile profiles revealed distinct differences in the number, composition and relative abundance of compounds among Chinnar, Karuppukavuni and Karunguruvai under healthy and BPH-infested conditions. Healthy Chinnar and Karuppukavuni exhibited two detected compounds each, whereas healthy Karunguruvai exhibited only one. Following BPH infestation, the number of detected compounds increased to four in Chinnar, five in Karuppukavuni and ten in Karunguruvai. These variations suggest differences in the chemical responses of the three varieties to herbivore attack, with Karunguruvai exhibiting the broadest range of detected compounds.

The varieties also differed in the dominant compounds identified following infestation. Chinnar exhibited a higher relative abundance of 2-butyl-2-ethyl-1,3-propanediol (6.76%) and 1-iodo-2-methylundecane (5.47%), whereas Karuppukavuni was characterised by n-butyl nitrite (16.91%) and 4,4,8-trimethyl-non-5-enal (14.06%). In Karunguruvai, 1,3-propanediol, 2-butyl-2-ethyl was the predominant compound, accounting for 16.63% of the total peak area. Notably, 1-iodo-2-methylundecane was detected in both Chinnar and Karunguruvai, while the dominant compounds differed considerably among the three varieties.

Table 3. Volatile profile of healthy and BPH-infested Karunguruvai, a traditional paddy variety

Name of the sample	RT	Name of the compound	Functional group	Peak area (%)
Healthy plants	10.13	(+)-2-Bornanone	Ketones	1.47
	20.11	1,2,3,4,5-Cyclopentanepentol	Alcohols	1.06
	21.93	Lactose	Minerals	0.76
	23.89	1-Iodo-2-methylundecane	Iodine-containing	2.57
	25.10	Diethyl phthalate	Esters	6.74
BPH-infested plants	25.64	Decane, 2,3,5,8-tetramethyl-	Hydrocarbons	2.23
	31.80	Dodecane, 2,6,10-trimethyl-	Hydrocarbons	3.34
	33.41	1,3-Propanediol, 2-butyl-2-ethyl	Alcohols	16.63
	40.36	1,2-Benzenedicarboxylic acid, butyl decyl ester	Esters	2.14
	41.20	Cyclic octaatomic sulfur	Sulfur	3.60

The observed variations are consistent with the broader understanding that herbivore-induced volatile emissions are influenced by plant characteristics and the nature of plant–herbivore interactions (Dicke & Baldwin, 2010). The emission of different volatile blends can contribute to indirect plant defence by mediating interactions between plants and the natural enemies of herbivores (Ali *et al.,* 2013; Degenhardt, 2009). The findings also reinforce the importance of considering the chemical specificity of plant volatile emissions when examining interactions between herbivores and their natural enemies (McCormick *et al.,* 2012). The occurrence of different classes of compounds, including alcohols, esters, aldehydes, hydrocarbons and iodine-containing compounds, further demonstrates the chemical diversity of the responses observed in these traditional rice varieties.

Overall, the findings indicate that Chinnar, Karuppukavuni and Karunguruvai exhibit distinct volatile profiles under healthy and BPH-infested conditions. The greater diversity of compounds detected in infested plants, particularly in Karunguruvai, and the differences in dominant compounds among the varieties provide important insights into the chemical responses associated with herbivore attack. These findings highlight the potential significance of volatile-mediated plant defence in traditional rice varieties and provide a foundation for further studies on the ecological functions of the identified compounds, particularly their roles in herbivore deterrence and the attraction of natural enemies. Such investigations could contribute to understanding the potential of traditional rice germplasm in developing ecologically sustainable approaches to rice pest management.

CONCLUSIONS

This research highlights the critical need to safeguard traditional crop varieties for their historical and cultural value and ecological significance in pest management. By enhancing our understanding of volatile organic compound emissions and plant defense strategies, this study advances more resilient agricultural systems, supporting food security and biodiversity preservation amid increasing environmental challenges.

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Ethics statement

Ethical approval was not applicable for this research experiment as only plant materials were used. Originality and plagiarism: the author confirms that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All sources used have been appropriately cited, and the manuscript has been prepared in compliance with plagiarism and publication ethics standards.

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Consent for Publication:

All authors agree to the content of the article and its publication in the journal.

Competing Interests

The author declares no competing interests.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request

Author Contributions

Meer Ahamed Ibrahim performed the experiments, did the analysis, and drafted the manuscript; Periyakaman Chandramani conceived and designed the research idea and supervised the experiments. Srinivasan Chinnadurai, Kuppusamy Nevashini and Saravanan Mohana contributed to the research coordination and interpretation of the findings. The manuscript was reviewed and revised collectively by all authors. All authors have read and approved the final version of the manuscript.

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