HERBIVORE-INDUCED PLANT VOLATILES IN RICE: A NATURAL DEFENSE MECHANISM SHAPING ARTHROPOD COMMUNITY

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Abstract. Rice plants have evolved defense mechanisms against insect attacks, leading to induced defense responses that can influence the composition of arthropod communities in rice fields. Inducing defense responses not only reduces rice pest population densities but also helps minimize the use of insecticides. Both herbivore-induced plant volatiles and elicitors are crucial in controlling insect pests and attracting natural enemies. The volatile profiles of insect-infested and uninfested rice plants were studied under screen house and open field conditions. GC MS/MS analysis of volatile samples of rice plants grown under screen houses and in the field revealed that at different growth stages of the crop (tillering, panicle initiation, and grain maturation), herbivore-infested rice crops contained a higher number of compounds (9 to 34 compounds) when compared to uninfested rice plants. The chemical profiles of infested and uninfested rice plants showed similarity in the presence of Eicosane, which was found in higher quantities in infested rice plants, particularly in the tillering stage. The composition of herbivore-induced plant volatiles varied depending on the growth stage of the rice crop. At the tillering and panicle initiation stages, Eicosane, Tetradecane, and Tridecane dominated the volatile blend in herbivore-infested plants. In contrast, at the grain maturation stage, Tetradecanoic acid and N-Hexadecanoic acid were more prevalent. The number of volatiles released by rice plants also varied across growth stages, with more compounds being released during the tillering stage compared to panicle initiation and grain maturation stages.
Keywords: *rice, plant volatiles, infested rice plant, uninfested rice plant, pests and natural enemies*

Introduction

Plants are repeatedly tackling different kinds of pests in their lifetime. These destructive pests use various strategies to subvert the plant's defensive mechanisms. By introducing various enzymes into the plant system, herbivores diminish the effect of plant toxins. The co-evolution of plants and pests has developed very intricate and efficient self-protection in plants, which helps in the survival of the plants during various biological stress situations (Balmer et al., 2013). Plant characteristics such as chemical, physical, and morphological barriers like cell wall lignification, trichome density, and silica deposition are responsible for induced direct defense (Howe and Jander, 2008). Plants enhance parasitization and predation by luring and keeping the natural enemies on a plant with shelters from harsh conditions, food rewards, chemical signaling, and/or prey availability (War et al., 2011). Herbivore oviposition fluids or oral secretions (OS) contain many elicitors that provoke plant defense responses, which may be present in the insect itself or derived from the plants while feeding (Lida et al., 2019). During an herbivory attack, the defense response elicitors are released, which

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provoke the volatile emission. These volatiles are perceived by the predators and parasitoids of the herbivore to trace their victims (Mattiacci et al., 1995).

Tritrophic interaction describes mutual communication amongst plants, herbivores, and natural enemies of herbivores such as predators and parasitoids (Price et al., 1980). Tritrophic interaction plays an essential role in sustaining a functional agroecosystem. Host plants emit a mixture of odor molecules called herbivore-induced plant volatiles (HIPVs) subsequent to the herbivore infestation that attracts natural enemies like predators and parasitoids (Guo and Wang, 2019). In nature, tritrophic interaction between plants, herbivores, and their natural enemies is intermediated by a complex range of stimuli, of which semiochemical plays a significant role (Mathur et al., 2012). HIPVs can initiate tritrophic interaction by attracting natural enemies towards the herbivore that is present on the plant (Ye et al., 2018).

Independent of visual contact, chemical signals can be perceived in the dark and can be diffused over distances ranging from short distances to long distances (Blassioli-Moraes et al., 2019). Plants use secondary metabolism-derived semiochemicals to protect themselves from herbivores (Bino et al., 2004). Herbivore-induced volatiles repel herbivores but at the same time attract natural enemies so that pest populations could be managed below damage levels (Smart et al., 2014). Semiochemicals can be incorporated with IPM strategies due to the heavy dependence of natural enemies on semiochemicals (Mathur et al., 2012).

From 900 plant families, about 2000 volatile compounds have been identified in response to herbivore infestations. Normally, plants emit a certain volume of volatile compounds into the atmosphere, but the chemical nature of these volatile compounds is altered during herbivore feeding (Kessler and Baldwin, 2002). To maintain a functional agroecosystem, tritrophic interaction plays an important role. Tritrophic interaction through elicitors and herbivore-induced plant volatiles (HIPVs) can be engaged in pest management strategies for effective crop production. The use of HIPVs as elicitors, release primers, or signalers of complete and correct blends of emissions that are attracting natural enemies is a striking possibility for manipulating parasitoid and predator populations in pest management (Khan et al., 2008). The present investigation was carried out to identify volatile profile differences in herbivore-infested and uninfested rice plants.

Materials and methods

Collection of volatile organic compounds (VOCs) in healthy rice plants at different growth stages in protected condition

Rice variety ASD 16 (medium tillering, semidwarf, erect, and white rice variety) was chosen for both screen house and field experiments. In the screen house, potted plants were maintained in the iron-meshed screen house (750 sq. ft.). Screenhouse rice plants were frequently observed to ensure the absence of herbivores.

Volatile collection unit

The volatile collection unit encompassed of a push-pull system. In this one, a battery-operated air pump was used to push air into the PET bag that contained the rice plants at a rate of 2 L/min. Another one was used to pull the air from the bag through the volatile collection trap (P/N: VCT - ¼- 4-HSQ-P Q:10 1/4” OD 4” L VCT), which was fixed at

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the outlet of the air pump. When the air and volatile mixture pass through the volatile trap, it will adsorb the volatiles present in the air. The collection of volatiles in the volatile trap was carried out for 3 h by a push-pull system (Kigathi et al., 2009).

Volatile collection from screen house rice plants

For the collection of plant volatiles from the screen house, four to five pots of rice plants were covered in a 50×50 cm polyethylene terephthalate (PET) bag that was devoid of noticeable traces of volatiles. The PET bag was cleaned using HPLC hexane (Merck) before the collection of volatiles. The volatiles were collected at three different stages, viz., tillering, panicle initiation, and the grain maturity phase (Plate 1). The volatile collection trap can be reused after cleaning to collect volatiles. These traps were cleaned with 5 ml of acetone and then dried at 240°C for 30 min. After cleaning, both ends of the trap were wrapped with aluminum foil to prevent contamination by outside air.

Collection of herbivore induced plant volatiles (HIPVs) in field condition

Volatiles were collected from herbivore-damaged rice fields by the above-mentioned method. Volatile collection was carried out in three different locations of Madurai district, Tamil Nadu, India, viz., Melur (10° 1’ 59.7288” N and 78° 20’ 9.0996” E), AC&RI, Madurai (9.9699° N, 78.2040° E), and Sholavandan (10.01980° N, 77.9720° E). In each location, volatiles were collected during tillering, panicle initiation, and the grain maturation stage of the rice (Plate 1). The symptoms and pests present in the field were recorded (Plate 2 and 3).

Extraction of volatiles from the volatile collection trap

The collected volatile organic compounds in the volatile trap were removed by eluting the absorbent with 20 ml of HPLC Hexane (Merck). Due to the higher affinity of the solvent for the absorbent medium, the collected volatile compounds displace solvent in place of volatiles.

Identification of volatiles in GC-MS/MS

The eluted extracts were concentrated by using nitro gas evaporator to 1 ml. The concentrated samples were filtered using syringe filter. The concentrated samples were characterized by using Gas chromatograph coupled with mass spectrometer (GC- MS/MS) (GC 2010 plus, GCMS – TQ 8040 SHIMADZU), in Central Instrumentation Laboratory, Centre of Innovation, Department of Entomology, Agricultural College and Research Institute, Madurai. The samples were filled in the glass vials and kept into an auto sampler (AOC-20i + s) for injection. The GC was fit with 30 m fused silica capillary column (Rxi 5Sil MS) with 0.25 mm ID and 0.25 µm film thickness. Volume of 1 µL sample was injected into GC-MS system using autosampler for analysis. The injection of sample was carried out through split less mode and the carrier gas helium were maintained at a constant flow rate of 1 ml/min. The oven temperature was programmed at 80°C and was held for 1 min to 100°C at a rate of 5°C/ min (1 min hold) then to 220°C at 10°C/min rate (5 min hold) and then to 240°C at 50°C/min (8 min hold). Injected sample was detected by mass spectrometer by separating the sample based on the retention time into various constituents.

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The detected volatile compounds were characterized using Shimadzu GC-MS lab solution software (Nishintha et al., 2019). The compounds of interest were identified using the NIST17 (National Institute of Standards and Technology) library. The identified compounds were tabulated along with the chromatogram, and a plot of intensity against retention time was recorded by the software attached to it. From the chromatogram, the compounds were identified by comparing the data with the existing software libraries.

Results

Volatile organic compounds (VOCs) in healthy rice plants at different growth stages in screen house condition

The volatile profile of healthy rice plants in screen house condition shows the presence of six, three, and six compounds during tillering, panicle initiation, and grain maturation, respectively. Eicosane was dominant in the tillering and grain maturation stages, with peak areas of 29.27 and 20.62, respectively. The least peak area (5.69) was occupied by 5, 5 diethyl hepta decane during the tillering stage. Whereas 2,6,11-trimethyldodecane was least during the grain maturation stage (5.53), whereas during the panicle initiation stage, 1-Nonadecene was dominant (48.18%), followed by 1-Iodo-hexacosane (14,87) and 1-Heneicosanol (24.72) (*Table 1*).

Herbivore induced plant volatiles (HIPVs) at different growth stages of rice

Tillering stage

In all three locations the yellow stem borer *Scirpophaga incertulas* (Walker) (Lepidoptera; Crambidae), green leaf hopper *Nephotettix virescens* Distant (Hemiptera; Cicadellidae) and leaf folder *Cnaphalocrocis medinalis* Gunee (Lepidoptera; Crambidae), were found to infest rice. In Melur, the crop was also infested by skipper *Pelopidas mathias* Fabricius (Lepidoptera; Hesperiidae) and in Solavandhan it was rice horned caterpillar *Melanitis leda* Linnaeus (Lepidoptera; Nymphalidae). The volatile profile of herbivore infested rice plants of three locations revealed the presence of 24, 39 and 31 compounds respectively. The common volatile profile was tridecane, tetradecane, pentadecane, octadecane and eicosane. Eicosane was predominant in AC&RI, Madurai and Solavandhan with the peak area of 13.52 and 9.71% respectively. In Melur, the predominant compound was hexacosane (19.087) and Eicosane had only 11.68% (*Table 2*).

Panicle initiation stage

In all three locations the infestation of leaf folder and stem borer was continued. In Melur, occurrence of brown plant hopper *Nilaparvata lugens* Stal (Hemiptera; Delphacidae) and black bug *Scotinophora sp* (Hemiptera; Pentatomidae) was noticed and in Solavandhan ear head bug *Leptocorisa acuta* Thunberg ((Hemiptera; Alydidae) infestation was recorded. The number of compounds identified at three different locations were 16, 23 and 20. Eicosane and tetradecane were the common compounds found in all three locations. Among which eicosane was higher in Melur and Solavandhan area (16.49 and 16.37 respectively) followed by AC&RI, Madurai (8.86). Next to eicosane another compound found in all three locations were Tetradecane and Pentadecane. Tridecane was found in AC&RI, Madurai and Melur (*Table 3*).

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At grain maturation stage

The infestation of *C. medinalis* and *S. incertulas* was continued in all three locations during grain maturation stage and *L. acuta* infestation was noticed in AC&RI, Melur. In Solavandhan, infestation of Yellow hairy caterpillar *Psalis pennatula* Fabricius (Lepidoptera; Erebidae) and cutworm *Mythimna separata* Walker (Lepidoptera; Noctuidae) were recorded. During grain maturation also eicosane was common in all locations and had similar peak area of 14.88. Tetradecane was registered at AC&RI, Madurai and Melur with similar peak area of 8.4%. Octadecane was found only in Melur (19.307) (*Table 4*). The number of herbivore induced volatile organic compounds was minimum at grain maturation stage.

Collectively, tridecane, 2,6,10-Trimethyldodecane, Tetradecane, Eicosane, n-Hexadecanoic acid, Tetrapentacontane, 2,6,11-trimethyldodecane, 11-Methyltricosane and Octadecane were the HIPVs present in all the three stages of the rice crop which was commonly infested with yellow stem borer, *S. incertulas*. Volatile blend of both tillering and panicle initiation stage of the rice crop detected with twenty one compounds in common *viz.*, 2-Methyltetradecane, 2-Methylhexacosane, Hexadecane, 2,6,10-Trimethyltridecane, 2,4-Dimethylundecane, 2,6,10-Trimethyltetradecane, Pentadecane, Octacosane, Heneicosane, 5-Methyloctadecane, 2,6,10,14-Tetramethylhexadecane, Dotriacontane, Tetracosane, 1-Hexacosene, Heptadecane, 2-Bromo dodecane, 1-Iodohexacosane, Octadecanoic acid, 2,6,10-Trimethyltetradecane, Tetradecanal and Octacosane. 1-Nonadecene and 2-Bromo tetradecane were present in tillering and grain maturation stage. Tetradecanoic acid, Nonadecane and 1-Iodo-dotriacontane were present in the volatile mixture of the rice crop at panicle initiation and grain maturation stages.

Discussion

Most of the identified VOCs from the uninfested and infested rice plants had carbon atoms ranging from 12 to 32. Among the identified compounds, Dodecane, 3,8-Dimethyldecane, 2-Bromododecane, and 4-Ethyldecane had the least number of carbon atoms (C12). Dotriacontane was a higher-order hydrocarbon with 32 carbon atoms. Rani and Sandhyarani (2012) reported volatile compounds with 10 to 30 carbon atoms were present in the yellow stem borer damaged stem extract. A volatile blend of 20–30 carbon atoms aids in the parasitization of *Trichogramma japonicum* (Rani et al., 2007). Paul et al. (2008) and Wolfling and Rostas (2009) reported that higher-order hydrocarbons were responsible for enhancing the attraction of parasitoids more than lower-order hydrocarbons.

Volatile samples of rice crops from both protected and field conditions revealed that qualitative and quantitative differences were found between healthy and damaged rice plants. A volatile blend of both uninfested and infested rice plants contains 2,6,11-Trimethyl dodecane, Eicosane, Tetradecanoic acid, n-Hexadecanoic acid, 1-Iodohexacosane, 1-Nonadecene, Tridecane, Hexadecane and 5,5-Diethyl heptadecane in common. Rani and Sandhyarani (2012) reported the presence of eicosane in both infested and uninfested rice crops. 1-Iodooctacosane and 1-Heneicosanol were present only in the uninfested, healthy rice plants. A higher peak area of volatiles was found in the volatile samples of damaged rice plants than in the uninfested, healthy rice crop (*Figs. 1–4*).

Table 1. *Volatile organic compounds (VOCs) in healthy rice plants at different growth stages in protected condition*

S.No.	Tillering stage				Panicle initiation stage				Grain maturation stage			
	VOC	RT (min)	% Area	Peak area	VOC	RT (min)	% Area	Area	VOC	RT (min)	% Area	Peak area
1.	2,6,11-Trimethyl dodecane	10.247	15.32	355352	1-Iodo hexacosane	14.872	20.87	262592	Tridecane	6.490	18.32	766941
2.	Eicosane	14.873	29.27	678879	1-Nonadecene	20.958	48.18	606114	Hexadecane	8.395	23.48	983117
3.	Tetradecanoic acid	16.479	25.65	594902	1-Heneicosanol	24.721	30.95	389406	2,6,11-Trimethyl dodecane	10.255	9.53	399099
4.	5,5-Diethyl heptadecane	18.667	5.69	132036					Eicosane	14.878	20.62	863477
5.	1-Iodooctacosane	20.155	8.09	187644					1-Nonadecene	16.881	18.46	772852
6.	N-Hexadecanoic acid	20.565	15.97	370427					1-Heneicosanol	24.733	9.59	401455

VOC – volatile organic compounds; RT – retention time

Table 2. *Herbivore induced volatile organic compounds in rice ecosystem growth stage: tillering*

S.No.	AC&RI, Madurai				Melur				Solavandhan			
		RT (min)	% Area	Peak area	VOC	RT (min)	% Area	Area		RT (min)	% Area	Peak area
1.	2,6,11-Trimethyldodecane	6.073	10.12	6626680	Dodecane	5.096	3.20	4764139	Tridecane	6.495	7.30	1617408
2.	Tridecane	6.495	6.04	3953840	Tridecane	6.492	9.13	13609924	3-Cyclohexyldecane	7.438	2.34	517657
3.	Heptadecane	7.675	2.67	1746344	4-Cyclohexyltridecane	7.432	2.39	3562256	2-Methyltridecane	7.666	1.86	411028
4.	2,6,10-Trimethyldodecane	7.902	9.71	6358522	2-Methyltetradecane	7.813	1.45	2159206	2,10-Dimethylundecane	7.805	2.05	453186
5.	Tetradecane	8.397	6.51	4262595	2,6,10-Trimethyldodecane	7.898	7.28	10852480	2,6,10-Trimethyldodecane	7.902	8.06	1785196
6.	Pentadecane	9.390	2.95	1935229	Tetradecane	8.391	6.90	10282358	Tetradecane	8.397	8.17	1808519
7.	2,6,10-Trimethyltridecane	9.640	3.98	2605083	2-Methylhexacosane	8.718	1.35	2005185	2,6,10-Trimethyltridecane	9.637	2.26	500217
8.	2-Bromo dodecane	9.696	3.94	2581823	Hexadecane	9.413	2.41	3587520	Heptadecane	9.694	2.61	576958
9.	2,4-Dimethylundecane	10.155	1.76	1153450	2,6,10-Trimethyltridecane	9.635	2.94	4377434	5,5-Dibutylnonane	10.145	1.00	220772
10.	Hexadecane	10.493	3.44	2254740	2,4-Dimethylundecane	9.692	3.91	5825888	2,6,10-Trimethyltetradecane	10.390	1.17	259092
11.	11-Methyltricosane	11.463	1.26	827957	2,6,10-Trimethyltetradecane	9.848	1.18	1764559	Pentadecane	10.494	2.85	631917
12.	Heneicosane	14.257	2.53	1656684	2,4-Dimethyldodecane	10.145	1.40	2079886	Hexadecane	12.683	3.91	864717
13.	Eicosane	14.874	13.52	8855430	Pentadecane	10.491	4.13	6153078	Tetrapentacontane	15.091	2.02	447086
14.	1-Tetracosene	16.883	1.02	666986	Octacosane	12.842	1.47	2185381	5-Methyloctadecane	15.735	3.64	805927
15.	Octadecane	17.016	1.69	1107644	2,6,10-Trimethylpentadecane	13.713	1.65	2461205	Eicosane	15.819	9.71	2149923
16.	1-Iodohexacosane	18.580	1.30	852397	Heneicosane	14.250	2.04	3041005	1-Heptadecene	16.873	2.78	615715
17.	Dotriacontane	18.669	2.74	1794352	2,3-Dimethylheptadecane	14.619	1.31	1945129	Octadecane	17.015	2.82	625097

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18.	2-Bromotetradecane	18.969	2.38	1559098	Eicosane	14.873	11.68	17411327	Heneicosane	18.665	4.58	1013397
19.	2-Methylhexacosane	19.551	2.17	1418504	5,5-Diethylpentadecane	14.950	2.04	3041535	Tetratetracontane	18.802	4.96	1098545
20.	Hexacontane	20.345	1.51	988969	5-Methyloctadecane	15.749	1.88	2807962	1-Bromo-2-methyl-decane	18.963	4.51	999463
21.	N-Hexadecanoic acid	20.577	11.02	7220726	1-Iodo-triacontane	15.923	1.36	2022383	1-Iodo-triacontane	19.137	1.99	439762
22.	Tetrapentacontane	23.370	3.47	2275481	2,6,11,15-Tetramethylhexadecane	16.015	0.93	1387367	2,3-Dimethyldodecane	20.315	1.04	229834
23.	3-Methylheptadecane	23.960	1.44	946075	1-Nonadecene	16.877	1.43	2134365	Octacosanol	20.954	0.67	148126
24.	Octadecanoic acid	24.356	1.32	861601	2,6,10,14-Tetramethylhexadecane	17.152	1.28	1911166	Hexacosane	21.077	3.44	761664
25.	1-Acetoxynonadecane	25.028	1.51	988856	11-Methylpentacosane	18.201	1.04	1543994	Tetradecanal	21.558	1.54	341845
26.					2-Bromotetradecane	18.965	2.53	3776694	Dotriacontane	23.369	5.85	1295047
27.					Hexacosane	19.087	1.37	2040070	Octadecanoic acid	24.354	0.86	190214
28.					Dotriacontane	19.299	4.63	6893483	1-Nonadecene	24.724	2.48	549612
29.					2,6,10,14-Tetramethyloctadecane	19.590	1.52	2267980	11-Methyltricosane	26.515	0.99	219664
30.					Hexadecanoic acid, methyl ester	19.673	0.96	1423330	13-Methylheptacosane	26.982	1.47	326448
31.					N-Hexadecanoic acid	20.578	6.25	9306344	Octacosane	28.266	1.08	238458
32.					Tetracosane	22.890	1.34	2002868				
33.					Tetrapentacontane	23.367	4.68	6979964				
34.					1-Hexacosene	24.727	0.94	1400706				

VOC – volatile organic compounds; RT – retention time

Table 3. *Herbivore induced volatile organic compounds in rice ecosystem growth stage: panicle initiation*

S.No.	AC&RI, Madurai				Melur				Solavandhan			
		RT (min)	% Area	Peak area	VOC	RT (min)	% Area	Area		RT (min)	% Area	Peak area
1.	4,6-Dimethyldodecane	6.003	8.84	730792	2,6,11-Trimethyldodecane	6.067	3.25	2899698	4-Ethyldecane	5.875	4.56	540591
2.	Tridecane	6.499	16.05	1326660	Tridecane	6.494	4.45	3976555	4,6-Dimethyldodecane	6.006	4.43	525749
3.	4-Cyclohexyldecane	7.435	5.16	426466	2-Methyltetradecane	7.810	1.59	1419996	Hexadecane	6.495	11.25	1334755
4.	2-Bromo dodecane	7.800	2.76	228313	2,6,10,14-Tetramethylhexadecane	7.901	6.51	5818920	Octadecane	7.672	2.51	297186
5.	2,6,10-Trimethyldodecane	7.905	10.71	885577	Tetradecane	8.397	7.44	6644038	3-Methyl-tridecane	7.810	2.57	304537
6.	Tetradecane	8.396	18.54	1532791	3,8-Dimethyldecane	9.400	3.41	3047620	2,6,10-Trimethyldodecane	7.904	10.96	1299756
7.	2,6,11-Trimethyldodecane	10.253	4.14	341845	2,6,10-Trimethyltridecane	9.637	3.89	3474525	Tetradecane	8.397	14.49	1719450
8.	Pentadecane	10.500	4.43	365936	2-Bromo dodecane	9.697	3.74	3340830	9-Methylnonadecane	9.695	5.21	617718
9.	Heneicosane	12.688	2.27	187423	2,6,10-Trimethyltetradecane	9.854	0.97	868548	2,4-Dimethylundecane	10.390	1.07	126530
10.	Eicosane	14.876	8.86	732324	3,7,11-Trimethyl-1-dodecanol	10.049	0.95	845179	Pentadecane	10.496	3.89	461282
11.	Tetradecanoic acid	16.480	5.60	462836	Heptadecane	10.395	1.66	1485789	Heptadecane	14.247	1.62	192193

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12.	3-Ethyl-2,6,10-Trimethylundecane	17.780	0.93	76499	Pentadecane	10.499	4.66	4166334	2-Methylhexacosane	14.764	2.20	260779
13.	5,5-Diethylheptadecane	18.670	1.58	130885	5-Methyloctadecane	14.325	0.92	819146	Eicosane	14.871	16.37	1942368
14.	N-Hexadecanoic acid	20.574	4.94	408153	Eicosane	14.875	16.49	14735106	Heneicosane	17.010	2.15	255359
15.	Nonadecane	22.994	2.91	240346	Heneicosane	17.015	2.59	2315509	Dotriacontane	18.665	2.54	301232
16.	2-Methylhexacosane	23.371	2.30	190055	Dotriacontane	18.668	4.32	3855724	Tetrapentacontane	19.293	5.87	695840
17.					Octacosane	18.812	5.47	4889953	N-Hexadecanoic acid	20.576	4.18	496038
18.					Nonadecane	19.085	2.78	2480480	Octacosane	21.084	2.17	257906
19.					1-Iodohexacosane	19.460	3.69	3298138	11-Methyltricosane	21.949	1.04	123714
20.					4,8,12-Trimethyltridecanoic acid	19.685	1.49	1329467	1-Iodo-dotriacontane	22.685	0.93	110115
21.					N-Hexadecanoic acid	20.580	7.24	6468253				
22.					Tetradecanal	21.561	1.34	1199657				
23.					9-Methylnonadecane	21.810	1.31	1168962				
24.					Pentatriacontane	22.450	0.79	702466				
25.					Tetracosane	22.883	1.53	1369187				
26.					Tetrapentacontane	23.365	4.48	4005326				
27.					Octadecanoic acid	24.353	0.87	773704				
28.					1-Hexacosene	24.726	2.17	1937636				

VOC – volatile organic compounds; RT – retention time

Table 4. *Herbivore induced volatile organic compounds in rice ecosystem growth stage: grain maturation*

S.No.	AC&RI, Madurai				Melur				Solavandhan			
		RT (min)	% Area	Peak area	VOC	RT (min)	% Area	Area		RT (min)	% Area	Peak area
1.	Tridecane	6.494	6.19	746894	2,6,10-Trimethyldodecane	6.080	14.94	529973	n-Tridecan-1-ol	12.524	1.86	185590
2.	2,6,10-Trimethyldodecane	7.910	3.80	458297	Tridecane	6.500	14.66	520198	Eicosane	14.871	5.06	505189
3.	Tetradecane	8.398	7.38	889734	Tetradecane	8.400	20.23	717758	2,6,11-Trimethyl dodecane	15.819	1.76	175640
4.	2-Methyldecane	10.504	1.16	140372	2,6,10,15-Tetramethylheptadecane	12.688	7.33	260137	Tetradecanoic acid	16.581	60.82	6074404
5.	2-Bromotetradecane	12.696	1.89	228430	Eicosane	14.881	23.26	825254	n-Heptadecanol-1	16.879	6.48	646898
6.	Eicosane	14.880	6.76	815841	1-Hexadecanol	16.875	3.66	129864	1-Iodo- dotriacontane	19.294	2.91	290707
7.	Tetradecanoic acid	16.603	61.25	7387158	Nonadecane	17.025	4.89	173628	n-Hexadecanoic acid	20.571	9.48	946364
8.	N-Hexadecanoic acid	20.583	9.12	1099419	Octadecane	19.307	5.89	209122	1-Nonadecene	20.959	5.97	596486
9.	Tetrapentacontane	23.378	2.44	294305	11-Methyltricosane	20.164	5.13	181862	n-Nonadecanol-1	24.722	3.51	350307
10.									Pentacos-1-ene	28.187	2.15	215146

VOC – volatile organic compounds; RT – retention time

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Figure 1. *Screen house rice plants for volatile collection*

Figure 2. *Collection of volatiles from screen house rice plants*

Figure 3. *Collection of volatiles at tillering stage*

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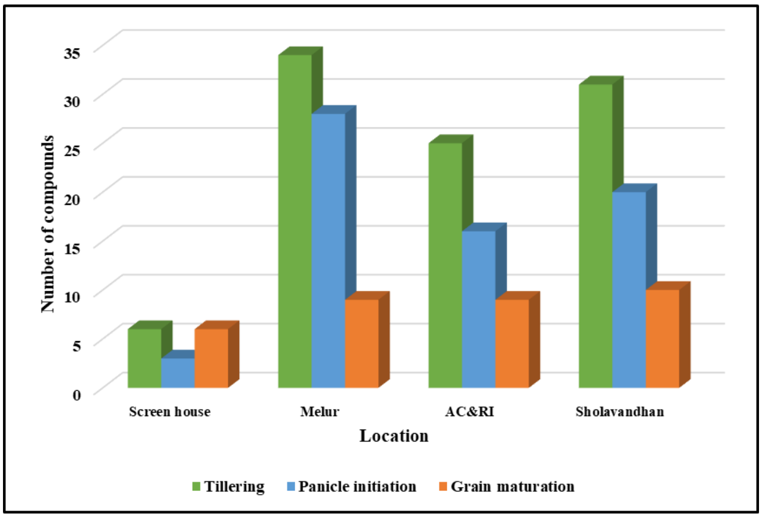

Figure 4. *Quantitative comparison of volatiles released from different stages of healthy and damaged rice plants at different locations*

The total number of compounds and quantity of volatiles were at their minimum in the undamaged plant. The Bt and non-Bt rice plants damaged by *C. supressalis* larvae released a wider range of volatile organic compounds than the uninfested Bt and non-Bt rice (Liu et al., 2015). Plants infested with herbivores release a much larger amount of volatiles (Dicke and Van Loon, 2000; Lu et al., 2014). Tao et al. (2002) reported that a minimum number of volatiles were detected from the undamaged, mechanically damaged, and brown planthopper, *N. lugens* (Stal.) damaged for 1 or 2 days, rice plants, than the brown planthopper damaged for 3 or 5 days, and also stated that rice leaves infested with *Spodoptera litura* emitted a greater number of volatiles than the healthy and mechanically damaged plants.

Conclusion

This study provides insights into the herbivore-induced plant volatiles present in rice crops at different growth stages, highlighting their potential role in influencing arthropod community composition and pest management. Understanding these defense mechanisms and their interactions with natural enemies can contribute to the development of sustainable and eco-friendly approaches for rice crop protection and pest control.

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