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GC-MS analysis for saturated hydrocarbons from rice varieties damaged by stem borer, *Scirpophaga incertulas* (Walker)

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ABSTRACT

The hexane extracts of two popular rice varieties viz., *ASD 16 and ADT 37 damaged by stem borer,* Scirpophaga incertulas *(Walker) and also healthy rice plants were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to determine the saturated hydrocarbons (SHC). The results revealed that both the healthy and damaged plants of rice variety ASD 16 had hydrocarbon compounds numbering 17 whereas both showed variation in the profile of the compound. In the variety ADT 37, healthy plants had 17 compounds whereas stem borer damaged plants contained 14 compounds. The variation in the hydrocarbon profile of healthy and damaged plants might be related to the quality of semiochemicals these plants emit, which is important for the attraction of natural enemies in the rice ecosystem so as to reduce the further infestation by stem borer. This feature can be exploited to enhance the efficacy of natural enemies in integrated pest management of rice crop.*

Key words: *Semiochemicals, saturated hydrocarbons, Scirpophaga incertulas, GC-MS, rice*

Rice is the world's most important food crop and is the staple food for 50% of the global population (Barrion *et al.,* 2007). The crop is attacked by several insect pests from nursery to harvest, which cause severe yield loss to the country (Asghar *et al.,* 2009). Yellow stem borer (YSB) *Scirpophaga incertulas* (Walker) has emerged as one of the most important pests of rice during post green revolution years throughout the country (Alam *et al,* 1992; Bandong & Litsinger, 2005; Krishnaiah *et al.,* 2008). It causes a yield loss of 10 million tonnes and accounts to 50 per cent of the insecticides used in the rice field (Huesing and English, 2004). An approach of using semiochemicals in pest management is to exploit ways to chemically augment or to conserve or enhance the efficacy of natural enemies in a crop ecosystem. Use of these semiochemicals especially, the synomones released by host plants is of significance in biological control. Hydrocarbons present in host plants were found to act as synomones for natural enemies in different crop ecosystems. In particular, synomones play a major role by guiding the natural enemies to the potential host or prey on the plant (Hilker and Meiners, 2006). Such clues may be utilized to stimulate foraging and host selection behaviour of entomophages thereby increasing their effectiveness for IPM (Ahmad *et al.,* 2004). Therefore, in the present study, the hexane stem extracts of two rice varieties *viz.,* ASD 16 and ADT 37 damaged by stem borer and also the healthy plants were analyzed through GC-MS to determine the saturated hydrocarbons profile in them.

MATERIALS AND METHODS

Two rice varieties *viz.,* ASD 16 and ADT 37 were planted at the Agricultural College and Research Institute, Madurai each with the plot size of 100 Sq.m during December-March cropping season of 2014-

2015. All the recommended agronomic practices for raising good crop were followed. Healthy and stem borer damaged plants of two rice varieties *viz.*, ASD 16 and ADT 37 were collected from the field during heavy infestation by stem borer.

The saturated hydrocarbons were extracted from the stem borer damaged and healthy stems using HPLC grade hexane as follows. The stems of the rice plants were isolated by carefully removing the leaves and roots at their bases. The remaining shoots (stems) of the plants infested with the stem borer and the stems of the normal intact and non infested plants were used for extraction. Thirty gram of stems were taken, washed in distilled water, air dried and immersed overnight in 300ml of HPLC grade hexane. The hexane extracts were then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @1g/10g sample was added to the filtrate for dehydration for 2 h and then it was passed through silica gel column and then distilled at 60 - 70^{0}C. The left over residue was collected using a small quantity of HPLC grade hexane (Merck) and kept in vials for GC-MS analysis (Archna kumar *et al.,* 2011).

Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. Hexane based stem extracts were analysed on a GC MS-QP 2010 Plus (Shimadzu, Kyoto, Japan) mass selective detector (70 eV) equipped with a 10 : 1 split injector. The gas chromatograph was equipped with 30 m fused silica capillary column having 0.25 mm ID and 0.25 μm film thickness run in constant flow mode (1.0 ml/min helium). Oven temperature programming: 60°C (1 min hold) to 100°C at 5°C/min rate (1 min hold), then to 220°C at 10°C/min rate (5 min hold) and then to 240°C at 50°C/min rate (8 min hold). Injector temperature was set at 275°C. One microlitre of the extract was injected using autosampler into the gas chromatography–mass spectrometry (GC–MS) system for analysis. Injections were done in split 10: 1 mode. Shimadzu GC-MS Lab solution software was used for the analysis of compounds in the extracts. Injected sample is separated into various constituents with different retention time which were detected by mass spectrophotometer. The compounds of interest were identified using standard NIST mass spectral (NIST MS 2) library. The chromatogram a plot of intensity against retention time was recorded by the software attached to it. From the graph, the compounds were identified by comparing the data with the existing software libraries.

RESULTS AND DISCUSSION

Induction of plant defense in response to herbivore involves the emission of volatile compounds called synomones that act as attractants for natural enemies of herbivores. Synomones produced by plants are reported to be very significant in eliciting host-seeking response in many natural enemies. Synomones attract predators and parasitoids which elucidate the tritrophic interaction in a crop ecosystem. Gas Chromatography- Mass Spectrometry analysis of synomone extracts of two rice varieties indicated that healthy plants of rice variety ASD 16 had 17 hydrocarbon compounds namely Cyclopentanol, 3-Hexanol, 2-Hexanol, Cyclohexanol, Decane, Octadecanal, 9,12,15-Octadecatrienoic acid, Hexadecanoic acid, Octadecane, 4-Cyclooctene-1-methanol, 2-hexadecen-1-ol, Docosane, Tricosane, Dotriacontane, Pentacosane, Hexatriacontane and Tetrapentacontane whereas stem borer damaged plants contained Cyclopentanol, 3-Hexanol, 2-Hexanol, Cyclohexanol, Decane, Tetradecanal, Octadecanal, 9,12,15-Octadecatrienoic acid, Hexadecanoic acid, 7,10-Hexadecadienal, 2-Hexadecen-1-ol, Docosane, Tricosane, Dotriacontane, Pentacosane, Hexatriacontane and Tetrapentacontane. In the variety ADT 37, Seventeen compounds were identified in healthy plants namely Cyclopentanol, 3-Hexanol, 2-Hexanol, Cyclohexanol, Decane, Tetradecanal, 1-Hexadecanol, 2-Pentadecanone, Tridecanal, 9,12,15-Octadecatrien -1-ol, 2-methyltetracosane and 9,12,15-Octadecatrienoic acid, Hexadecanoic acid, 2-Hexadecen-1-ol, Tetrapentacontane, Tricosane and Pentacosane and in case of stem borer damaged plants, 14 compounds were identified *viz.,* Cyclopentanol, 3-Hexanol, 2-Hexanol, Cyclohexanol, Decane, 2-Pentadecanone, Tetradecanal, 9,12,15-Octadecatrienoic acid, Hexadecanoic acid, 2-Hexadecen-1-ol, Tetrapentacontane, Tricosane, Pentacosane and Dotriacontane. These results are in accordance with the findings of Kumar *et al.* (2012) who reported that the hexane leaf extracts of targeted cole crop variety, White Vienna subjected to gas liquid chromatography to detect their saturated hydrocarbon profile revealed the presence of three favourable hydrocarbons *viz.*, heneicosane (C21), docosane (C22) and nonacosane (C29) (Table 1).

Comparing the peak areas of hydrocarbon compounds present in healthy and stem borer damaged plants, eleven compounds had more peak area in damaged than healthy and four compounds had more peak area in healthy than damaged plants in the variety ASD 16. In the variety ADT 37, ten compounds had more peak area in healthy and only three compounds had high peak area in damaged stem extracts.

In ASD 16, two compounds *viz.*, 7, 10-hexadecadienal and tetradecanal were present only in damaged plants whereas octadecane and 4-Cyclooctene-1-methanol were found in healthy plants and not in damaged plants. Tridecanal, 1- Hexadecanal, 9,12,15-Octadecatrienoic acid and 2-methyltetracosane were present only in healthy plants whereas dotriacontane was present only in damaged plants and not in healthy plants of ADT 37 (Table 2).

Seenivasagan and Paul (2011) analyzed the extracts of cruciferous host plants of diamond back moth and revealed the presence of saturated hydrocarbons. Cauliflower leaf extract contained 12 hydrocarbons with carbon number ranging from C10-C30 in which C29 (nonacosane) was detected in highest quantity. In cauliflower extracts exclusively C10 (Decane) and C12 (Dodecane) hydrocarbons were identified which were not detected in other host plant extracts. The hydrocarbon C14 (Tetradecane) was detected only in cauliflower and broccoli extracts, whereas C16 (Hexadecane) was detected only in cabbage, cauliflower and broccoli extracts. C18 (Octadecane) and C20 (Eicosane) were detected in cabbage and cauliflower extracts. C22 (Docosane) and C25 (Pentacosane) were detected only in cauliflower; while, C26 (Hexacosane) was found only in knol khol leaf extracts. Similarly, hexane extracts of ten different varieties of tomato (*Lycopersicon esculentum* Mill) obtained in the vegetative and flowering phase of growth contained tricosane (C23), heneicosane (C21), pentacosane (C25) and hexacosane (C26) during the vegetative period and heneicosane (C21), hexacosane (C26) during the flowering period (Paul *et al.*, 2008).

Thus to summarize, stem borer damaged plants had more peak area when compared to the healthy rice plants. In ASD 16, the hydrocarbon compounds *viz.*, Octadecanal, 9,12,15- Octadecatrienoic acid, hexadecanoic acid, 2-Hexadecen-1-ol had 4.18, 3.82, 2.71 and 2.06 times more peak area in stem borer damaged plants. In the variety ADT 37, the hydrocarbon compounds namely tetradecanal, tetrapentacontane and

Table 1. Saturated hydrocarbon profile of the healthy and stem borer damaged plants of ASD16

Healthy plants			Stem borer damaged plants		
RT	Area	Name	RT	Area	Name
3.218	32289637	Cyclopentanol	3.222	35079809	Cyclopentanol
3.308	19065350	3-Hexanol	3.311	22565334	3-Hexanol
3.353	9134504	2-Hexanol	3.359	10698334	2-Hexanol
4.711	1162386	Cyclohexanol	4.715	1038830	Cyclohexanol
7.720	152197	Decane	7.725	209522	Decane
			16.903	1694512	Tetradecanal
18.078	1828786	Octadecanal	18.058	7640781	Octadecanal
19.904	5289169	9,12,15-Octadecatrienoic acid	19.896	20187054	9,12,15-Octadecatrienoic acid
20.954	984069	Hexadecanoic acid	20.953	2665394	Hexadecanoic acid
21.155	491554	Octadecane			
21.556	556309	4-Cyclooctene-1-methanol			
			21.550	4288855	7,10-Hexadecadienal
22.098	2489747	2-Hexadecen-1-ol	22.104	5129482	2-Hexadecen-1-ol
22.992	1870112	Docosane	22.992	1124432	Docosane
23.924	9401329	Tricosane	23.926	11912616	Tricosane
25.014	3090855	Dotriacontane	25.012	1244861	Dotriacontane
26.342	5951134	Pentacosane	26.345	7049166	Pentacosane
27.965	2020542	Hexatriacontane	27.977	1068216	Hexatriacontane
30.020	6105918	Tetrapentacontane	30.031	10459454	Tetrapentacontane

Table 2. Saturated hydrocarbon profile of the healthy and stem borer damaged plants of ADT37

Healthy plants			Stem borer damaged plants		
RT	Area	Name	RT	Area	Name
3.218	60853569	Cyclopentanol	3.216	32584561	Cyclopentanol
3.308	47021867	3-Hexanol	3.305	21990159	3-Hexanol
3.352	20676545	2-Hexanol	3.357	9783781	2-Hexanol
4.710	1851580	Cyclohexanol	4.708	1333460	Cyclohexanol
7.719	238560	Decane	7.719	196167	Decane
16.894	1348823	Tetradecanal	18.058	8190721	Tetradecanal
17.657	880361	1-Hexadecanol			
17.883	846033	2-Pentadecanone	17.887	1890340	2-Pentadecanone
18.054	10642186	Tridecanal			
18.839	3063003	9,12,15-Octadecatrien -1-ol			
19.151	346238	2-methyltetracosane			
19.892	27485101	9,12,15-Octadecatrienoic acid	19.899	24706922	9,12,15-Octadecatrienoic acid
20.950	1706837	Hexadecanoic acid	20.956	858003	Hexadecanoic acid
22.102	5308958	2-Hexadecen-1-ol	22.110	2710916	2-Hexadecen-1-ol
23.460	2869188	Tetrapentacontane	23.464	10713358	Tetrapentacontane
23.922	9369938	Tricosane	23.925	4674124	Tricosane
26.340	5433848	Pentacosane	26.350	3606089	Pentacosane
			30.020	7147736	Dotriacontane

2-pentadecanone had 6.07, 3.73 and 2.23 times more peak area in stem borer damaged plants than healthy rice plants. Therefore, the hydrocarbon compounds found in the extracts of two rice varieties *viz.*, ASD 16 and ADT 37 have to be explored for the attraction of natural enemies to parasitize and/or to predate the herbivore or reduce the further infestation by the herbivores for efficient pest management.

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