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Volatile profile of yellow stem borer, *Scirpophaga incertulas* (Walker) damaged rice plants

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Abstract

A monophagous pest of rice, Yellow stem borer (*Scirpophaga incertulas*) is the most destructive among the rice pests. It accounts for 50 % of all the insecticides used in rice field. The persistent and injudicious use of chemicals have toxic effects on non-target organisms and can cause undesirable changes in the environment, thus aiming for an alternative method of pest management, studies were carried out in tritrophic interaction (Herbivore Induced Plant Volatiles). The GCMS analysis of the volatile profile of Yellow stem borer infested rice plants indicated the presence of Decane, Dodecane, Undecane, Pentadecane, Hexacosane, Octacosane, Nonadecane, Eicosane, Octadecane, Heneicosane, Tridecane, Tetradecane, Tridecanol, 1- Tridecene and Triacontane that are responsible for the attraction of natural enemies.

Keywords: Rice, yellow stem borer, herbivore induced plant volatiles, GC- MS/ MS

Introduction

Rice (*Oryza sativa*) is the staple food for more than 65 percent of the total population of the world and accounts for more than two third of Indian population. It is cultivated in 112 countries all over the globe and being consumed by 2500 million people in the developing countries. Among the constraints in the rice production, insects pests remain as the major limiting factors of rice that caused 20 to 30 % losses every year (Visalakshmi *et al.*, 2014) [10]. The two important lepidopteran pests in rice ecosystem are Rice leaf folder and Yellow stem borer that cause severe damage to the rice crop.

A monophagous pest of rice, Yellow stem borer (*Scirpophaga incertulas*) is the most destructive among the other rice pests due to its abundant distribution and chronic pattern of infestation (Deka and Barthakur, 2010) [3]. It causes 3 to 95 % yield losses in India which is higher loss than any other insect pest of rice and also accounts for 50 % of all the insecticides used in rice field (Huesing and English, 2004) [5]. The persistent and injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Most of these chemicals are too expensive for the resource poor rice farmers.

The natural defence of the plants may act directly or indirectly against pest. The direct defence by the plants is by affecting the host plant preference or survival and reproductive success which is mediated by mechanical protection or by secondary metabolites. The indirect defence against insects are induced by the release of blend of volatiles that may attract natural enemies. Plants emit volatile and non-volatile compounds to defend themselves from herbivore attack. Herbivore-induced plant volatiles (HIPVs) play an important role in plant defence by either attracting the natural enemies of the herbivores or by acting as feeding and/or oviposition deterrent to the pest. The HIPVs include terpenes, green leafy volatiles (GLVs), ethylene, methyl salicylate and other Volatile Organic Compounds (VOCs). HIPVs are involved in plant communication with natural enemies of the insect herbivores, neighbouring plants and different parts of the damaged plant (Abdul Rashid *et al.*, 2012) [1].

The chemical signal from plant is exploited by both pest and natural enemies for foraging the food. The induced plant volatiles play a major role in alteration of plant interaction with environment. The current scenario of research on metabolomics, proteomics and genomics studies need biochemical information of plant volatiles. Hence the present study is undertaken with tritrophic interaction (HIPVs).

Materials and Methods

Plant culture

The recent popular Tamil Nadu rice variety CO – 51 was cultured in trays for 14 days that was maintained in a chamber and were transferred into pots in separate cages. These potted plants were used for volatile collection.

Insect culture

Mass culturing of Yellow stem borer, *Scirpophaga incertulas* was done for obtaining uniform population. Adult moths were collected with a sweep net during day time. The collected moths were kept in an ovipositional cage containing 40 – 50 days old rice seedling and were fed with 10 per cent sucrose solution soaked in water. After, oviposition, the egg masses were collected by cutting the leaf section containing egg. The leaf section along with the eggs were placed in a petri dish lined with moist filter paper and were stored in room temperature until the end of black headed stage. The hatched larvae were used for further studies.

Chemicals and equipments

The Volatile collection chamber and Volatile collection trap – Tenax – Q™ filled 3” long borosilicate tube was procured from Mascot Enterprises.

The extraction of plant volatiles was carried out using Hexane (HPLC Grade) as solvent that was obtained from Sigma Aldrich chemicals, the same solvent was used for Head space analysis of the collected volatiles. The glass vials with – PTFE/ Silicone septa silver screw caps from Agilent Technologies were used for volatile collection. The maximum internal pressure of this vial is 310 KPa.

Volatile collection

The herbivore induced plant volatiles were collected using a volatile collection unit. Initially air was pumped into the air tight glass chamber for about 45 minutes and then vacuum was created inside it. The stem borer larvae were released into the potted plants (CO – 51) that were cultured in cages and were kept inside an air tight glass jar. Air was pumped into the jar through activated charcoal at a rate of 100 ml/ min. The herbivore induced volatiles from the stem borer damaged rice plants were trapped in a volatile trap (absorption tube) (Gainesville, FL (352) 283- 0133; P/N: VCT - ¼- 4-POR-Q:1/4” OD × 4” L VCT) that was attached to glass jar. The volatiles were collected for 3 hours by pumping the air into

the glass jar. The volatiles being adsorbed were extracted using 20 ml HPLC grade Hexane.

Adsorption tubes were cleaned with acetone (5 ml) and then dried at 240 °C for 30 minutes and then covered using aluminium foil to avoid contamination by outside air. The cleaned tubes were reused for the collection of volatiles.

Headspace analysis

Head space analysis was carried out using Gas chromatograph coupled with mass spectrometer (GC- MS/MS) (GC 2010 plus, GCMS – TQ 8040 SHIMADZU). The collected volatiles (1 ml) were loaded in glass vials of capacity 20 ml. The GC was equipped with 30 m fused silica capillary column (Rxi® - 624 Sil MS) having 0.25 mm ID and 0.14 µm film thickness. The injection was carried out in splitless mode and the helium gas flow was maintained constant at 1 ml/ min (Annamaria *et al.*, 2012). 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 rate (8 min hold). The oven temperature was maintained at 80 °C. The analysis of the collected volatiles was carried using Shimadzu GC-MS Lab solution software. The identification of compounds fragmented was performed by NIST14 (National Institute of Standards and Technology). The spectrum of the unknown compounds were identified by comparing with that of the known compounds in the library.

Results and Discussion

The volatile profile of the CO – 51 rice plants due to stem borer attack showed the presence of hydrocarbon volatiles. The elicited volatiles compounds by GC-MS/MS analysis are Decane, Dodecane, Undecane, Pentadecane, Hexacosane, Octacosane, Nonadecane, Eicosane, Octadecane, Heneicosane, Tridecane, Tetradecane, Tridecanol, 1 – Tridecene and Triacontane. Certain compounds of this profile were also present in the healthy plants but in limited quantity as compared to plants infested by stem borer. These compounds are responsible for the attraction of natural enemies in rice plants. Triacontane accounts 11.9 % of the total compounds detected. This was followed by Octacosane with a per cent area of 11.54. Dodecane (7.77%), Eicosane (5.65%), Hexacosane (3.67%) were also detected. Undecane (0.51%) and 1-Tridecene (0.23%) had the least peak areas.

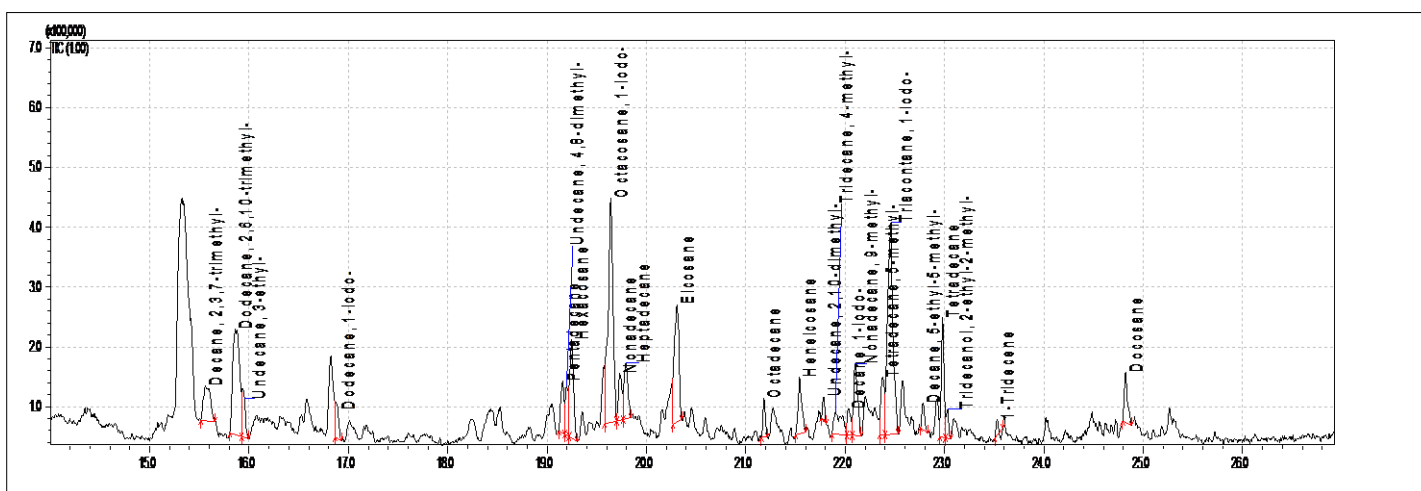


Fig 1: Chromatographic profile of volatile compounds from Yellow stem borer, *Scirpophaga incertulas* infested rice plant.

In fig. 1, X axis represents the retention time and Y axis represents the intensity.

Table 1: Volatile profile detected in the headspace of rice plants infected with Yellow stem borer, *Scirpophaga incertulas*.

S. No.	Retention Time	Area	Compound name
1.	15.561	287667	Decane, 2,3,7-trimethyl-
2.	15.862	858850	Dodecane, 2,6,10-trimethyl-
3.	15.955	174369	Undecane, 3-ethyl-
4.	16.895	127603	Dodecane, 1-iodo-
5.	19.154	200555	Pentadecane
6.	19.195	200755	Undecane, 4,8-dimethyl-
7.	19.246	405509	Hexacosane
8.	19.639	1275555	Octacosane, 1-iodo-
9.	19.730	204387	Nonadecane
10.	19.790	251131	Heptadecane
11.	20.306	624042	Eicosane
12.	21.182	103235	Octadecane
13.	21.540	239682	Heneicosane
14.	21.783	55872	Undecane, 2,10-dimethyl-
15.	21.900	225263	Tridecane, 4-methyl-
16.	22.03	109663	Decane, 1-iodo-
17.	22.098	324264	Nonadecane, 9-methyl-
18.	22.37	255117	Tetradecane, 5-methyl-
19.	22.458	1314741	triacontane, 1 – iodo-
20.	22.782	85948	Decane, 5-ethyl-5-methyl-
21.	22.98	379720	Tetradecane
22.	23.03	78116	Tridecanol, 2-ethyl-2-methyl-
23.	23.525	25795	1-Tridecene
24.	24.82	184585	Docosane

Deepa and Bakthavatsalam (2012) ^[4] indicated that the presence of Heptadecane, Nonadecane and Octadecane are responsible for the attraction of egg parasitoid, *Trichogramma japonicum*. Madhu *et al.*, (2000) ^[7] indicated that nonadecane in sorghum elicited higher parasitization by *T. japonicum*. The different rice varieties tested for volatile profile (HIPV's) showed relevant compounds that were responsible for the attraction of natural enemies (Pavviya *et al.*, 2018) ^[8]. In sugarcane, *Cotesia flaviceps* (parasitoid) showed behavioural response to the shoot borer damaged plants. This is due to the volatiles released by the plants due to the damage caused by the lepidopteran borers (Salin *et al.*, 2016) ^[9]. Lou *et al.*, (2005) ^[6] reported that *Anagrus nilaparvatae*, an egg parasitoid of *Nilaparvata lugens*, (Rice brown planthopper) did not prefer volatiles from healthy plants instead it preferred volatiles emitted by plants damaged by *N. lugens*.

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