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# EFFECT OF SILICON AMENDMENT ON HERBIVORE INDUCED PLANT VOLATILES OF RICE PLANT INFESTED BY BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STÅL)

Prabhulinga Tenguri[+], Subhash Chander[1*], Suresh Nebapure, Arya P S, Madhu T N[2+] and Yogesh Yele

Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI);
[1]ICAR-National Research Centre for Integrated Pest Management (NCIPM), New Delhi 110012, India
[2]Department of Entomology, University of Agricultural Sciences (UAS), GKVK, Bengaluru 560065, Karnataka, India
[+]Current Address: ICAR-Central Institute of Cotton Research, Nagpur 440010, Maharashtra, India
*Email: schanderthakur@gmail.com (corresponding author)

**ABSTRACT**

**Silicon (Si) is known to play a very important role in a plant's direct and indirect defense. In rice plants, its impact on induced volatiles released upon brown planthopper (BPH) *Nilaparvata lugens* feeding is less understood. The BPH-induced volatile compounds from Si amended rice plants were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The alteration in Herbivore Induced Plant Volatiles (HIPV's) blend was observed, wherein, total 38 HIPVs were found to be differentially released. The HIPV mainly belongs to alkane, alkene, alcohol and terpene groups. Overall, Si amendment caused a significant effect on the composition of HIPVs in rice, some of which are involved in tritrophic interaction.**

**Key words:** GCMS, herbivore, induced plant defense, Pusa Basmati 1121, rice, semiochemical, silicon, TN1, tritrophic interaction, volatile organic compounds

Rice *Oryza sativa* L. is one of the important cereal crops, and its production is affected by biotic and abiotic stresses. Among these, insect pests are the major ones, and around 52% of the production is lost annually because of the biotic factors, of which insects pests contribute nearly 21% (Khush, 1979; Sogawa et al., 2003). Brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) is the important insect pests of rice, which damages the crop directly by its typical phloem sap-feeding and indirectly by transmitting virus diseases such as rice grassy stunt and ragged stunt (Cabauatan et al., 2009). In recent years in Asia, the rice production has been threatened by the BPH infestation (Brar et al., 2009; Prasannakumar et al., 2013; Prahalada et al., 2017). The management of this pest has relied upon insecticides; however, their indiscriminate use disrupts the natural balance of rice ecosystem (Sarao and Mangat, 2014; Prahalada et al., 2017). In order to minimize the negative effects of insecticides, use of safe alternatives is required. Silicon (Si) application is one such safe alternative, and Si is a quasi-essential nutrient. The evidence from recent investigation reveals its roles in plant defense against biotic and abiotic stress (Epstein,1994; Luyckx et al., 2017; Cooke and Leishman, 2016) including against insect herbivores and pathogens in agriculture (Ye et al., 2013; Wang et al., 2017). Si application enhances the plant defense by increasing the rigidity of the plant by increasing the deposition of Si on the plant surface and by inducing the production of defense chemicals (Yang et al., 2017). Also, the Si application indirectly enhances the plant defense by altering the composition of herbivore induced plant volatile (HIPV) which acts as synomones (Becker et al., 2015) to which natural enemies such as predators and parasitoids get attracted that act on the herbivores (Mumm and Dicke, 2010; Schuman et al., 2012). The present study evaluates the effect of Si application on the compositional changes of HIPVs in rice induced by BPH feeding.

## MATERIALS AND METHODS

BPH population was collected from rice fields of ICAR- Indian Agricultural Research Institute (IARI), New Delhi (28$^o$38'N, 77$^o$09'E). The population was reared on rice varieties viz., TN1, and Pusa Basmati 1121 in the glasshouse with optimum rearing conditions of 27+2$^0$C, 75%± 5% relative humidity and 14 hr light/10 hr dark photoperiod. The established population was further used for the experiments. The rice variety, Pusa Basmati 1121, which is susceptible to *N. lugens,* was used in the investigation and its nursery was raised by following all the package of practices. The 21 days old seedlings were transplanted in pots (25x 22 cm) filled with silicon treated soil following seedling root

Table 1. Relative abundance (% of total peak area) of volatile organic compounds (VOC) in Si treated Pusa Basmati 1121 rice after BPH infestation

| S. No. | VOCs | Functional group | % of total peak area of VOCs in Si-treated plant | % of total peak area VOCs in without Si-treated plants |
|---|---|---|---|---|
| 1 | D-Limonene | Monoterpene | 0.66± 0.04 | 0.49± 0.01 |
| 2 | (*E*)-2-Hexanol | Alcohol | 2.51± 0.11 | 1.92± 0.14 |
| 3 | Dodecene | Alkene | 0.31± 0.02 | 0.35± 0.01 |
| 4 | b-linalool | Monoterpenoid | 3.2± 0.14 | 2.87± 0.12 |
| 5 | Nonane | Alkane | 0.47± 0.01 | 0.41± 0.02 |
| 6 | Docosane | Alkane | 0.53± 0.01 | 4.56± 0.45 |
| 7 | Cyclopentane | Cycloalkanes | 1.85± 0.23 | 3.93± 0.36 |
| 8 | Tetradecane | Alkane | 2.42± 0.12 | 0.28± 0.01 |
| 9 | Hexadecane | Alkane | 0.74± 0.03 | 0.33± 0.11 |
| 10 | Toluene | Aromatic hydrocarbon | 7.65± 1.2 | 3.78± 0.23 |
| 11 | Eucalyptol | monoterpenoid | 0.49± 0.05 | - |
| 12 | Mesitylene | Aromatic Hydrocarbon | 7.7± 0.97 | 4.4± 0.50 |
| 13 | Z-8-Octadecen-1-ol acetate | Alcohol | 0.21± 0.01 | 0.46± 0.02 |
| 14 | Benzene, 1-ethyl-2-methyl- | Toluene | 2.5± 1.1 | 2.12± 0.74 |
| 15 | 1,2-Benzenedicarboxylic acid | Phthalic acid | 1.12± 0.47 | 1.08± 0.33 |
| 16 | Undecane | Alkane | 0.66± 0.11 | 0.75± 0.17 |
| 17 | o-Xylene | Arene | 6.0± 0.98 | 3.75± 0.36 |
| 18 | n-Eicosane | Alkane | 0.20± 0.01 | 0.37± 0.02 |
| 19 | Benzene, propyl- | Arene | 0.36± 0.04 | 0.21± 0.01 |
| 20 | a-cedrene | Sesquiterpene | 0.04± 0.01 | - |
| 21 | Benzene, 1,2-dichloro- | Arene | 1.73± 1.0 | 1.74± 0.54 |
| 22 | 2-Piperidinone | piperidine | 0.34± 0.01 | 0.31± 0.01 |
| 23 | 1-Decanol | Alcohol | 0.68± 0.03 | 0.75± 0.11 |
| 24 | Naphthalene | Alkene | 15.54± 2.12 | 3.36± 0.98 |
| 25 | 3-Carene | Monoterpene | 0.4± 0.01 | - |
| 26 | (+)-2-Bornanone | - | 0.57± 0.21 | - |
| 27 | Spiro [3.5]nona-5,7-dien-1-one, 5,9,9-trimethyl- | | 0.1± 0.04 | 0.15± 0.03 |
| 28 | Heptane | Alkane | 0.48± 0.01 | 0.36± 0.07 |
| 29 | 7-Oxabicyclo [2.2.1] heptane | - | 0.21± 0.02 | 0.32± 0.01 |
| 30 | 2-Propyl-1-pentanol | Aliphatic alcohol | 2.07± 0.45 | 1.89± 0.66 |
| 31 | Benzene, 1,3-diethyl- | Aromatic hydrocarbon | 6.93± 1.21 | 7.23± 1.74 |
| 32 | Benzene, 1,3-diethyl- | Aromatic hydrocarbon | 9.0± 2.30 | 6.79± 2.11 |
| 33 | 3-Heptyne-2,6-dione, 5-methyl-5-(1-methylethyl)- | - | 1.84± 0.11 | 1.55± 0.25 |
| 34 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | - | 1.08± 0.02 | 1.47± 0.31 |
| 35 | 17-Pentatriacontene | Paraffin Hydrocarbon | 0.23± 0.02 | 0.21± 0.01 |
| 36 | alfa.-Copaene | Sesquiterpene | 0.12± 0.02 | 0.08± 0.01 |
| 37 | Dichloroacetic acid, 6-ethyl-3-octyl ester | - | 0.48± 0.04 | 1.02± 0.01 |
| 38 | 2-Pentanone, 4-hydroxy-4-methyl- | Ketone | 0.45± 0.11 | 0.73± 0.09 |

treatment carbendazim @ 0.2%. As a source of silicon, calcium silicate (CaSiO3) (≥ 87% SiO2 and 12-22% CaO) was used in two concentrations i.e., 0 g and 0.32 g Si/ kg soil. After transplanting the pots were caged with (mylar sheet) and kept in a glasshouse until further use. The BPH nymphs ($2^{nd}$-$3^{rd}$ instar) were transferred to caged rice plants at 10:1 insects/ plant. The volatile organic compounds (VOC) released from Pusa Basmati 1121 rice were collected by the dynamic headspace collection (Liu et al., 2017) for the experimental treatments: +Si +Herbivore; +Si -Herbivore; -Si +Herbivore; and -Si -Herbivore. Porapak Q (80-100 mesh; Sigma-Aldrich) was used as a volatile adsorbent. VOCs collection was started after 24 hr post infestation (hpi) from 60 days old seedlings and done for 5 hr and then eluted using 300 µl dichloromethane (DCM). The internal standard nonyl acetate (100 ng/ µl) was added DCM. Elute was analysed through GC-MS (Shimadzu QP 2000). The oven temperature was held at 40°C for 3 min then increased at 5°C to 220°C $min^{-1}$. Compounds were identified by using the mass spectra with the inbuilt library (NIST 14).

## RESULTS AND DISCUSSION

Thirty-eight identified compounds from the volatile blend belongs to alkane (nonane, docosane, tetradecane, hexadecane, undecane, n-eicosane, heptane, cyclopentane,), alkene (dodecene, naphthalene), ketone (2-pentanone, 4-hydroxy-4-methyl-), alcohol ((*E*)-2-hexanol, Z-8-octadecen-1-ol acetate, 1-decanol, 2-propyl-1-pentanol), terpenes (D-limonene, b-linalool, eucalyptol, a-cedrene, 3-carene, alfa - copaene), aromatic hydrocarbons (toluene, mesitylene, 1,3-diethyl-benzene, 1-ethyl-3-methyl- benzene) and arene, paraffin hydrocarbon, phthalic acid and toluene groups were found to be released (Table 1). However, the composition of volatile blends was affected by *N. lugens* infestation as also reported earlier (Lou et al., 2005). Some of these volatiles emitted by plants after herbivore damage play the roles of semiochemicals (Pare and Tumlinson, 1999; Degenhardt et al., 2003), which are involved in tritrophic interaction (Vet and Dicke, 1992; Lou et al., 2005). Many of the HIPVs recorded from Si treated and untreated plants are the same except for the differences in their relative abundance.

It has been observed earlier those parasitoids of leaf folder *Cnaphalocrocis medinalis* had greater attraction towards the blend of HIPVs produced by plants without Si treatment (Liu et al., 2017). The role of these HIPVs in tri-trophic interactions needs to be studied further. Silicon is the second most abundant element in the earth's crust, but the majority of it is in unavailable form (Ma and Yamaji, 2006). However, many investigations have proved that it is required in large quantity in available form viz., silicic acid [Si(OH)4] or Si(OH)3$O^-$. The significance of Si to the plants defense against biotic and abiotic stresses has been shown in many crops. Especially, in rice, the Si has shown direct defense by increasing the rigidity of the plant tissue and indirectly by inducing the production of defensive chemicals (Yang et al., 2017). However, the change in volatile compounds composition in Si treated plants upon *N. lugens* infestation is unexplored. In this study we could identify and compare the HIPVs of +Si and –Si plants, some of which are having semiochemical properties. Further, for more understanding, the role of important HIPVs against natural enemies of BPH needs to be studied by using olfactometer tests.

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## AUTHOR CONTRIBUTION STATEMENT

PT, SC, SN conceived and designed research. PT, APS, and YY conducted experiments. PT, SN, and MTN analyzed data. PT and SN wrote the manuscript. All authors read and approved the manuscript.

## CONFLICTS OF INTEREST

Authors declares that there is no conflict of interest

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