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ORIGINAL CONTRIBUTION

Soil application of silicon reduces yellow stem borer, *Scirpophaga incertulas* (Walker) damage in rice

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Keywords
imidazole, larval midgut, mandibles, rice husk ash, *Scirpophaga incertulas*, white ears

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Abstract

The effect of soil application of rice husk ash (RHA), a cheap renewable source of silicon, and imidazole (a silicon solubilizer and carrier) on yellow stem borer (YSB), *Scirpophaga incertulas* (Walker), and its damage to rice plants were investigated. Treatments included soil application of RHA (T1), imidazole (T2), RHA + imidazole (applied once at vegetative stage, T3) and RHA + imidazole (applied twice at both vegetative and booting stages of crop growth, T4) with an untreated control (T5). The effect was tested in five varieties, viz., BPT 5204, KRH2, Pusa Basmati 1, MTU1010 and Vandana. All the soil treatments reduced damage by YSB at vegetative and reproductive phases across five varieties as compared to untreated control. Scanning electron micrograph and electron-dispersive X-ray spectrum analysis of stem tissue of rice variety BPT 5204 treated with silicon revealed the enhanced deposition of silicon in cell walls and 2.1- to 5.3-fold increase in silicon content across treatments. Larvae collected from the silicon-treated plants had worn mandibles, and the histological studies showed rupture of the peritrophic membrane, increased vacuolation, disintegration of columnar cells and discharge of cellular contents into the gut lumen due to abrasion of midgut epithelium, as compared to untreated control where the columnar cells and midgut lining were intact. Although all the treatments were effective, T4-imidazole applied twice along with RHA was more effective in reducing YSB damage followed by T3-imidazole applied once with RHA at tillering stage, T2-imidazole alone and T1-RHA in the descending order of efficacy.

Key message

- Rice husk ash, a natural silicon source, and imidazole, a solubilizer and carrier of silicon, were studied for their effect on yellow stem borer damage in rice.
- Soil application of silicon reduced the damage by yellow stem borer in the five rice varieties at vegetative and reproductive phases of crop growth.
- Soil application of silicon enhanced the deposition of Si in the rice stem as evidenced by scanning electron microscopy and electron-dispersive spectrum studies.
- Decrease in weight was observed in larvae that fed on silicon-treated varieties. High larval mortality was observed in imidazole-treated plants. Morphometric studies revealed the wearing of the mandibular incisors. The larval midgut integrity was altered due to abrasion by silicon and affected the histology.

Introduction

The yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae), is the predominant species of rice stem borer throughout the rice-growing regions in Asia and South-East Asia (Khan et al. 1991) and attacks all stages of the rice crop (Bandong and Litsinger 2005). Larval entry into tillers

during the vegetative phase leads to death of the growing shoots referred to as 'dead hearts', whereas during the reproductive phase, it causes unfilled panicles commonly known as 'white ear heads'. The yield loss may vary from 10% to 90% (Pathak and Khan 1994; Muralidharan and Pasalu 2006) depending on the stage of the crop when the insect attacks. Even after the repeated application of insecticides, it is difficult to manage YSB because of its cryptic behaviour. So there is always a search for viable alternative strategies to manage this pest. Application of silicon (Si) sources for the management of insect pests is one such option, although they need to be applied in large quantities and their effect on soil physical and chemical properties and soil biota is not known. Si can be applied through many inorganic sources (Anderson and Sosa 2001; Keeping and Meyer 2002; Correa et al. 2005; Goussain et al. 2005), bagasse furnace ash or fly ash (Keeping and Meyer 2006), sodium silicate (Na_2SiO_3; Basagli et al. 2003; Moraes et al. 2004), potassium silicate (K_2SiO_3; Subbarao and Perraju 1976; Parrella et al. 2007), calcium silicate (McCray et al. 2011) or organic sources in the form of rice husk ash (RHA; Savant et al. 1994) for the management of insect pests. Silicon solubilizers that increase the availability of silicon could be the best alternatives as they are organic in nature with no residual impact on soil health (Voleti et al. 2009) and are effective at very low doses. Application of Si solubilizers such as simple amino acids such as histidine, glutamic acid, glycine, glutamine and small biomolecules such as imidazole in rice (*Oryza sativa* L.) reduced damage by YSB and blast (*Pyricularia grisea*) (Ranganathan et al. 2006; Voleti et al. 2008). Of the many biocompatible molecules, imidazole ($C_3H_4N_2$), an organic, aromatic heterocyclic compound of the diazole group was found to be effective in enhancing the uptake of Si. Imidazole has a strong hydrogen bonding potential that can polarize the surface Si, promoting hydrolysis. This factor, in addition to the fact that the silicic acid formed can be stabilized by hydrogen bonding, promotes the equilibrium in favour of silicic acid. This leads to an enhanced solubilization, as the Si–water interface harbours an equilibrium largely consisting of Si and small amounts of the hydrolysed product, silicic acid. Imidazole promotes the equilibrium in favour of silicic acid by a dual mechanism involving favouring Si hydrolysis and its subsequent stabilization by hydrogen bonding (Voleti et al. 2009; Ranganathan et al. 2011). Thus it acts as both a solubilizer and carrier. RHA (henceforth referred to as RHA) is a potential source of silicon and is a cheaply available form of silicon (Prakash et al. 2007). Application of black-to-grey RHA at 0.5–1.0 kg/m^2 to the seedbed resulted in healthy and strong rice seedlings which tolerated stem borer damage (Savant et al. 1994).

In this study, we report the efficacy of a natural source of Si (RHA) and a solubilizer and carrier for Si (imidazole) alone and, in combination, against YSB incidence and damage in rice plants. The effect of transported/deposited Si on histology of larval midgut and its physical effect on mandibles were also investigated, thereby elucidating the mechanism involved in both the reduced growth of YSB and damage to rice plants.

Materials and Methods

All the experiments were carried out at ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad, India (17° 10′N and 78° E, 542 MSL), during 2011–2012.

Insect culture

Adult female YSB moths that were available for a specific period in a crop season were collected daily during early morning hours (6–9 am) from pesticides-free IIRR rice fields. The collected moths were released for egg laying onto potted plants of the rice variety, TN 1, covered by mylar cage. The pots were maintained at 28 ± 2°C temperature and 70% relative humidity in the greenhouse. Once the egg masses were laid, they were retained on the plants until the black head stage. The egg mass were then detached from the plants and placed in vials for the larvae to hatch. Neonate larvae that hatched from the egg mass were released onto the plants for experimental purpose. The experiments were planned in such a way that all the plants in the treatments were infested at the same time.

Varieties tested

The effect of Si vis-a-vis stem borer damage was studied on five most commonly grown varieties in different regions of India namely the following: BPT 5204, MTU 1010, KRH 2, Vandana and Pusa Basmati 1 (Table S1) at two stages of crop growth *viz.,* maximum tillering stage and booting stage in pot experiments.

Treatments

Rice husk from a single source was sun-dried and burnt to greyish black colour in earthen pots, powdered and sieved (60 mesh size). The powdered ash

was used for all the studies. The quantification of silicon present in RHA was carried out by the molybdenum yellow method (Saito et al. 2005). Imidazole (chemical formula: $C_3H_4N_2$, Mol. Wt – 68.08 from Hi-Pure Ine Chem Industries, Chennai, India) was dissolved in water and applied to soil. Treatments comprised of only RHA (T1), only imidazole (T2), RHA+ imidazole applied once at maximum tillering (MT) stage (T3), RHA+ imidazole applied twice, at both MT and booting stage (T4), and an untreated control (T5) (Table S1). The rate of application of imidazole was equivalent to 60.4 mg of Si/kg soil per application, and RHA was equivalent to 85 mg Si/kg soil to assess the effect on stem borer damage (Table S1) according to Voleti et al. (2008).

Pot experiments and treatment imposition

Seedlings (25–30 days old) of test rice varieties were raised in pots (18.5 cm diameter and 16 cm height) filled with five kg well-puddled soil mixed with vermicompost along with NPK at 100–60–40 kg/ha. Each test variety was planted in five hills per pot at the rate of two seedlings per hill in each pot. The experimental design was a factorial randomized block design with four replications.

Before the application of any soil treatments, plant samples were collected from all the varieties for the estimation of initial Si content in the stem tissues. All the treatments were applied to soil at 30 days after transplanting (DAT) by dissolving in a small quantity of water for proper distribution.

Seven days after application (7DAA) of soil treatments, freshly hatched neonate YSB larvae (10 larvae per pot) were released near the auricle of the top leaf of the plant in all the treatment combinations. After inoculation, the pots were kept apart giving adequate space to avoid migration of larvae from one pot to another pot. The experiments were repeated in both wet season, 2011, and dry season, 2011–2012.

Observations recorded

Stem borer damage

The damage was assessed by counting the number of dead hearts and the total number of healthy tillers in each pot at 3, 7, 10, 14 and 21 days after larval infestation (DAR) at vegetative phase of the crop. Pre-harvest count of white ears and the total number of panicle bearing tillers were recorded.

Per cent dead hearts and white ears were derived as follows:

$$\text{Per cent dead hearts} = \frac{\text{Total number of dead hearts}}{\text{Total number of tillers}} \times 100,$$

$$\text{Per cent white ears} = \frac{\text{Total number of white ears}}{\text{Total number of panicle bearing tillers}} \times 100.$$

Observations on larvae recovered from treatments

Plants were uprooted from pots of each treatment in each variety separately and thoroughly washed in running tap water to remove the adhered soil. The stems of plants were dissected out to collect the YSB larvae that remained at the base of the plant. Observations were recorded on the total number of live larvae and dead larvae from each treatment, and per cent mortality was calculated for each treatment. Immediately after collecting the larvae, weight of the live larvae was recorded in each treatment. After recording the weight, the larvae were preserved in 70% ethyl alcohol, for mandibular studies and larval midgut microtomy.

Effect of Si on larval midgut

This study was carried out to observe the effect of Si treatments on histology of larval midgut. From the larvae collected from each of the treatments, midgut of a larva was dissected out on a wax tray. The procedure given by Armed Forces Institute of Pathology (1960) was followed for larval microtomy to observe the effects of larval feeding on Si-treated stems.

Morphometry of larval mandibles

Studies were carried out to observe the effect of RHA and imidazole on larval mandibles due to feeding on rice varieties. The mandibles were dissected out from the head capsule of each larva (n = 15 per replication). Both left and right mandibles were separated and placed on a slide. The width (L1) at the base of the mandible and length (L2) of both left and right mandibles, that is from the base of the mandible to tip of the incisor region (Figure S1), were measured from the dorsal side at 630x using a Scopetek image analyzer as described by Raupp (1985).

Estimation of Si content in the stem tissues

Biochemical method

The total amount of Si present in the stems in all the test varieties where treatments were given was

estimated by the Molybdenum yellow method (Saito et al. 2005). Silicon was estimated in the stem tissues 1 day before application (1DBA), 7DAA and seven days after booting (7DAB) stage of crop growth. Time to booting depends on a rice variety.

Scanning electron microscopy

Scanning electron microscopy (SEM) studies were carried out on only rice stem of BPT 5204 variety during wet season, 2011. Stem tissue 5 cm above the ground was sampled at 60 DAT from each of the treatments for SEM study. Stem samples were cut into 2 cm sections, wiped with tissue paper to remove moisture, taped to aluminium stubs and taken for SEM study.

Samples from the stem were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C and post-fixed in 2% aqueous osmium tetroxide for 4 h. Later, the samples were dehydrated in a series of graded alcohol and dried to a critical point with a CPD unit. The processed samples were mounted on stubs with double-sided carbon conductivity tape, and a thin layer of gold coat was kept over the samples using an automated sputter coater (JOEL JFC-1600) for 3 min and scanned under scanning electron microscope (JOEL-JSM 5600) at required magnifications as per the standard procedure given by Bozzola and Russell (1999).

Electron-dispersive spectrum analysis for silicon deposition

Stem tissue of all treatments of BPT 5204 at 60 DAT in wet season was subjected to Electron-dispersive spectrum (EDS) study. Stem tissue 5 cm above the ground (as this is the major port for the larval entry) was sampled, and SEM was carried out using SEM SU1510 (Hitachi High technologies, Japan) to identify areas of Si deposition and then, elemental Si content in the stem tissue was quantified through electron-dispersive X-ray spectroscopy **(**EDAX, Thermo Scientific, USA).

Statistical analysis

All the statistical analyses were carried out in SAS® 9.2 (SAS® 2003) statistical software. General linear model procedure was adopted for all statistical analyses. Before analysis, data in percentages were arc-sine transformed. For bio-efficacy studies such as per cent dead heart at 3, 7, 10, 14 and 21 days after release of larvae (DAR) and per cent white ear, repeated measures ANOVA was carried out. Per cent larval mortality, larval weight, mandibular wear and silicon content were analysed using the factorial randomized block design (ANOVA) technique followed by Tukey's HSD test ($\alpha = 0.05$) for multiple comparisons.

Results

Bio-efficacy studies

Efficacy of RHA and imidazole on damage by YSB in the test varieties at 3, 7, 14 and 21 days after release (DAR) of larvae and at harvest was observed in wet season (table 1) and dry season (Table S2).

During wet season, 2011, 3 days after release of neonates in the soil treatments T4, T3 and T2, there was a delay in the formation of dead hearts (Figure S2a). Among soil treatments, significant differences were observed with respect to per cent dead heart and white ear. T4-RHA+ imidazole (twice) was the most effective treatment with least per cent dead heart and per cent white ear damage followed by T3-RHA +imidazole (once), T2-imidazole and T1-RHA (table 1). Similar trends were observed at 7, 14 and 21 DAR and also for white ear damage. Untreated control showed maximum per cent dead heart (62.2 ± 5.6) and white ear (31.1 ± 5.3) damage at 21 DAR. Significant differences were observed among varieties with respect to per cent dead hearts across all the time intervals, that is 3, 7, 14 and 21 DAR during wet season, 2011. Among varieties, PB 1 had the highest and KRH 2 the lowest dead heart damage (table 1).

At reproductive phase, the per cent white ear damage (Figure S2b) in T4-RHA+ imidazole (twice) recorded was the lowest followed by T3-RHA+ imidazole (once), T2-imidazole, T1-RHA and T5-control which were significantly different from each other (table 1). Among varieties, lowest per cent white ear damage was noticed in BPT 5204 followed by MTU 1010 and KRH 2, which were similar, and the highest was in PB 1, which was significantly different from other varieties. The interaction effects, DAR × varieties and DAR × silicon treatments were significant. However, DAR × varieties × silicon treatment interaction was non-significant (table 1). Similar trends were observed in the dry season, 2011–2012 (Table S2).

Observations on larvae recovered from soil treatments

Larval mortality

During wet season (2011) and dry season (2011–2012), the results indicated that, among soil treatments, the maximum per cent larval mortality was observed in T4-RHA+ imidazole (twice) which was significantly different from T3-RHA+ imidazole (once)

Table 1 Effect of soil treatments on per cent damage by YSB as influenced by varieties in greenhouse experiments, wet season, 2011

Varieties	Per cent dead hearts (Mean ± SD)[1]					Mean per cent white ears[1] ±SD
	3 DAR	7 DAR	10 DAR	14 DAR	21 DAR	
BPT 5204	1.4 ± 3.6	14.4 ± 4.9 a	21.1 ± 6.1 ab	26.7 ± 6.2 b	31.6 ± 5.0 b	14.9 ± 4.1 ab
MTU 1010	1.4 ± 4.3	13.9 ± 4.6 a	18.7 ± 3.0 a	23.7 ± 2.1 a	28.1 ± 2.9 a	15.7 ± 2.6 a
KRH 2	1.5 ± 3.5	11.7 ± 3.6 a	17.1 ± 2.9 a	24.4 ± 6.0 a	31.7 ± 3.0 a	16.1 ± 3.7 a
PB 1	2.6 ± 4.8	18.7 ± 3.6 b	28.5 ± 9.6 c	34.5 ± 8.4 c	37.9 ± 7.1 c	18.7 ± 3.6 c
Vandana	2.0 ± 3.9	17.9 ± 5.3 a	24.6 ± 6.1 ab	29.8 ± 6.2 b	34.4 ± 5.0 b	17.6 ± 4.2 b
Soil treatments						
T1-Rice husk ash (RHA)	1.7 ± 3.9 b	19.6 ± 5.0 c	29.2 ± 7.5 b	34.3 ± 8.7 c	39.3 ± 7.3 d	23.9 ± 5.1 d
T2-Imidazole	0.0 a	11.5 ± 3.9 b	17.6 ± 8.8 b	23.4 ± 9.6 b	28.0 ± 7.1 c	14.9 ± 3.3 c
T3-RHA+ imidazole (once)	0.0 a	8.3 ± 3.6 b	12.4 ± 5.7 a	18.1 ± 6.4 a	23.0 ± 5.4 b	10.6 ± 4.1 b
T4-RHA+ imidazole (twice)	0.0 a	6.2 ± 3.5 a	9.9 ± 5.1 a	14.7 ± 6.3 a	18.7 ± 4.7 a	7.6 ± 2.1 a
T5-Untreated control	15.1 ± 5.0 c	39.6 ± 6.3 d	49.8 ± 9.6 c	56.5 ± 6.9 d	62.2 ± 5.6 e	31.1 ± 5.3 e

Sources of variation	d.f.	F value	P value
DAR	4, 75	471.26	<0.001
Varieties	4, 75	10.77	<0.001
Soil treatments	4, 75	89.79	<0.001
Interaction effects			
DAR × varieties	16, 75	2.98	0.02
DAR × soil treatments	16, 75	4.39	0.003
Varieties × soil treatments	16, 75	1.58	0.09
DAR × varieties × soil treatments	64, 75	0.74	0.74

In a column, means followed by the same letter do not differ significantly from each other by Tukey's HSD ($\alpha = 0.05$).

[1]Mean values of per cent dead hearts and white ears from four replications for each treatment; DAR, days after release of larvae.

and T2-imidazole, and the least larval mortality was observed in T5-control and T1-RHA (table 2). Varieties had no effect on larval mortality.

Larval weight

Among the soil treatments, the lowest mean larval weight (mg) was noticed in T4-RHA+ imidazole (twice), which was similar to T3-RHA+ imidazole (once) and T2-imidazole, followed by T1-RHA (table 3). Maximum mean larval weight (mg) was recorded in control and significantly different from other treatments. The mean weight (mg) of larvae collected from the varieties did not differ significantly. During the dry season also the soil treatments had similar significant effects on the larval weight (table 3).

Effect of Si on larval midgut

Histological effects of silicon were highly pronounced in the larval midgut of YSB that were recovered from plants with the soil treatment (Figure S3). Midgut sections of the larvae that were obtained from the T1-RHA treatment showed slight alterations in the histology of epithelium (Figure S3A). It was observed that compactness was lost between columnar cells because of damage to connective tissue, and cells were ruptured to release the cytoplasmic granules into the lumen. The peritrophic membrane was completely ruptured, and increased vacuolation was observed in midgut sections of larvae collected from imidazole-treated plants (Figure S3B). Columnar cells were distended and ruptured, and cell granules were released into the lumen. The upper epithelial lining remained completely detached from cuticular basement due to abrasion in the midgut sections of the larvae exposed to a combination of RHA and imidazole treatments (Figure S3C and S3D). The peritrophic membrane was completely detached and destroyed. The cellular granules were discharged due to rupturing of columnar cells. Disorganization of epithelial cells was noticed. In contrast to soil treatments, larval midgut sections from the control treatment showed compact columnar cells, intact peritrophic membrane and no vacuolation (Figure S3E).

Morphometrics of larval mandibles

In the wet season, statistically significant differences were noticed in average length of incisor cusps (table 4). The maximum length of incisor cusps was noticed in larvae from untreated control, followed by

Table 2 Effect of soil treatments on YSB larval mortality as influenced by varieties

	Per cent larval mortality (mean ± SD)[1]											
	Wet season, 2011						Dry season, 2011–2012					
Treatments	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana	Mean	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana	Mean
T1-Rice husk ash (RHA)	22.3 ± 6.0 c	14.6 ± 4.1 d	22.3 ± 5.2 c	16.6 ± 6.0 c	24.4 ± 3.3 c	14.3 ± 4.3 c	19.3 ± 5.2 c	17.2 ± 4.4 c	17.2 ± 4.4 c	19.3 ± 5.2 c	22.3 ± 3.6 c	17.5 ± 4.1 c
T2-Imidazole	24.7 ± 3.0 b	27.3 ± 3.3 c	23.0 ± 4.7 c	21.3 ± 5.2 b	24.4 ± 5.2 b	24.8 ± 4.0 b	29.6 ± 3.6 b	27.3 ± 5.2 b	29.6 ± 3.6 b	29.6 ± 3.6 b	31.9 ± 4.3 b	30.1 ± 3.8 b
T3-RHA+ imidazole (once)	25.8 ± 5.1 b	26.9 ± 5.2 b	26.2 ± 3.5 b	24.7 ± 5.2 b	29.6 ± 6.0 b	26.5 ± 4.3 b	32.4 ± 4.3 b	29.6 ± 3.6 b	29.6 ± 4.1 b	29.6 ± 3.6 b	30.0 ± 3.6 b	30.7 ± 4.2 b
T4-RHA+ imidazole (twice)	37.1 ± 3.0 a	34.8 ± 4.8 a	30.0 ± 5.2 a	32.4 ± 5.7 a	31.9 ± 3.5 a	32.4 ± 4.6 a	37.4 ± 2.8 a	39.7 ± 5.5 a	34.8 ± 3.6 a	37.0 ± 4.3 a	34.8 ± 4.7 a	35.6 ± 4.8 a
T5-Untreated control	19.3 ± 4.0 d	10.0 ± 4.7 e	14.6 ± 4.1 d	14.6 ± 4.1 c	12.2 ± 4.7 d	14.1 ± 4.7 c	17.2 ± 4.4 c	14.6 ± 3 c	14.6 ± 3.0 d	17.2 ± 4.3 c	14.6 ± 2.8 d	15.6 ± 3.7 d
Mean	25.5 ± 6.1	21.7 ± 7.4	22.9 ± 6.8	21.5 ± 6.8	23.9 ± 6.3		26.6 ± 5.1	24.9 ± 4.6	24.5 ± 3.7	26.0 ± 5.2	26.2 ± 4.6	

	Wet season, 2011			Dry season, 2011–2012		
Sources of variation	d.f.	F value	Pr > F	d.f.	F value	Pr > F
Varieties	4	0.32	0.86	4	0.35	0.85
Soil treatments	4	30.43	<0.0001	4	31.24	<0.0001
Interaction effect (varieties × soil treatments)	16	0.73	0.73	16	0.72	0.74
Error	72			72		

In a column, means followed by the same letter do not differ significantly from each other by Tukey's HSD ($\alpha = 0.05$).

[1]Mean ± SD values of per cent larval mortality from four replications for each treatment.

Table 3 Effect of soil treatments on YSB larval weight (mg) as influenced by varieties

	Larval weight (mg)[1] (mean ± SD									
	Wet season, 2011					Dry season, 2011–2012				
Treatments	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana
T1-Rice husk ash (RHA)	22.3 ± 2.6 b	26.5 ± 1.7 b	25.0 ± 0.8 b	26.5 ± 1.0 b	23.8 ± 3.1 b	27.8 ± 1.6 b	28.0 ± 2.5 b	28.8 ± 1.7 b	29.5 ± 4.1 b	28.8 ± 1.5 b
T2-Imidazole	19.8 ± 2.9 c	19.3 ± 4.3 c	20.3 ± 1.3 c	21.0 ± 3.9 c	18.5 ± 3.0 c	21.8 ± 2.1 c	21.5 ± 1.5 c	23.3 ± 0.6 c	24.3 ± 2.6 c	21.5 ± 2.2 c
T3-RHA+ imidazole (once)	18.8 ± 3.6 c	18.3 ± 2.4 d	20.0 ± 2.1 c	20.0 ± 0.5 c	18.5 ± 2.5 c	19.3 ± 1.9 c	19.8 ± 2.3 c	19.8 ± 2.9 d	21.5 ± 3.5 d	19.8 ± 2.6 c
T4-RHA+ imidazole (twice)	18.5 ± 3.4 c	17.8 ± 3.9 d	17.5 ± 3.3 d	18.0 ± 1.3 d	16.8 ± 2.2 d	16.3 ± 2.3 d	17.0 ± 2.6 d	15.5 ± 3.0 e	19.3 ± 3.2 e	16.3 ± 2.1 d
T5-Untreated control	43.5 ± 5.8 a	44.0 ± 3.8 a	42.8 ± 2.1 a	44.0 ± 5.9 a	46.0 ± 1.8 a	42.0 ± 3.2 a	43.3 ± 2.1 a	40.8 ± 2.8 a	42.5 ± 2.6 a	41.3 ± 3.2 a
Sources of Variation	d.f.	F value	Pr > F	SEd±		d.f.	F value	Pr > F	SEd±	
Varieties	4	5.69	0.11	0.96		4	1.95	0.11	0.84	
Soil treatments	4	258.58	<0.0001	0.96		4	280.87	<0.0001	0.84	
Interaction effect (varieties × soil treatments)	16	0.84	0.99	2.15		16	0.35	0.99	1.87	
Error	72					72				

In a column, means followed by the same letter do not differ significantly from each other by Tukey's HSD ($\alpha = 0.05$).

[1]Mean ± SD values of weight of larvae recovered from four replications for each treatment.

T1-RHA, T2-imidazole, T3-RHA+ imidazole (once) and T4-RHA+ imidazole (twice) which were significantly different from each other (table 4). In our study, rice varieties had no effect on incisor length (table 4). During dry season, 2011–2012, similar trend was observed among larvae collected from soil treatments, with respect to average length of incisor cusps (table 4). In both the seasons, mandibular width was not influenced as it is a characteristic of the larval instar rather than the effect of Si (data not shown).

Estimation of Si content in the stem tissues

Biochemical method

During wet season, 2011, an inherent variation was observed between the varieties in the Si content (mg/g) in the stem tissue at 1DBA of treatments. The maximum Si content was noticed in KRH 2 followed by MTU 1010, and the least was in PB 1. Among the soil treatments, no significant difference in Si content was observed at 1DBA, and it varied from 29.7 to 30.1 mg/g (table 5). Seven days after the treatment imposition (7DAA), there were significant differences in the Si content in the stem tissue among the varieties tested where the maximum was recorded in KRH 2 and the lowest in PB 1. Among the soil treatments, the maximum Si content of 76.2 mg/g was recorded in T4-RHA+ imidazole (twice), followed by T3-RHA+imidazole (once) and T2-imidazole (table 5). The interaction effects were non-significant. Similar trend was noticed at 7DAB stage. Among soil treatments, highest Si content was recorded in T4-RHA+ imidazole (twice), followed by T3-RHA+ imidazole (once) and T2-imidazole, which were significantly different from each other (table 5). Among the varieties, the maximum Si content was recorded in KRH 2, and the lowest was in PB 1. Non-significant differences were noticed in interaction effects. Similar trend was observed in dry season (table 5).

SEM and EDS analysis of silicon deposition

The images of SEM (Figure S4) and EDS analysis (Figure S5) of elemental Si content in the stem tissues indicated different levels of Si accumulation in various soil treatments. SEM images of rice tissue samples clearly depicted the rows of dumb-bell-shaped cells of Si in all the soil treatments including T1-RHA, when compared to untreated control. Enhancement of Si content of stem tissue through soil treatment was observed through the images of EDS analysis. Elemental Si content (per cent by weight) was maximum in T4-RHA+ imidazole (twice) (19.0 ± 0.07%), followed by T3-RHA+ imidazole (once) (16.6 ± 0.10%),

Table 4 Effect of soil treatments on incisors length of YSB larval mandible as influenced by varieties

	Length of incisor cusps (L2) in YSB larval mandibles (μm)[1] (mean ± SD)											
	Wet season, 2011						Dry season, 2011–2012					
	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana	Mean	BPT 5204	MTU 1010	KRH 2	PB 1	Vandana	Mean
T1-Rice husk ash (RHA)	250.5 ± 9.0 b	251.0 ± 10.5 b	244.5 ± 9.4 b	250.0 ± 9.6 b	247.2 ± 7.4 b	248.6 ± 8.5 b	246.2 ± 11.2 b	244.0 ± 10.1 b	246.2 ± 6.9 b	250.5 ± 8.5 b	246.7 ± 8.2 b	246.7 ± 6.4 b
T2-Imidazole	230.2 ± 4.8 c	224.0 ± 5.5 c	226.0 ± 7.9 c	227.2 ± 0.9 c	227.2 ± 4.3 c	226.9 ± 7.4 c	223.7 ± 6.7 c	219.7 ± 6.6 c	228.5 ± 5.6 c	228.2 ± 6.0 c	228.5 ± 4.1 c	225.7 ± 8.1 c
T3-RHA+ imidazole (once)	220.0 ± 4.5 d	216.0 ± 7.8 d	211.5 ± 9.3 d	213.0 ± 8.3 d	219.5 ± 9.3 d	216.0 ± 8.2 d	214.5 ± 3.3 d	216.0 ± 7.1 d	213.5 ± 6.0 d	219.7 ± 4.2 d	216.0 ± 5.9 d	215.9 ± 7.6 d
T4-RHA+ imidazole (twice)	201.5 ± 10.3 e	197.7 ± 6.2 e	202.0 ± 10.1 e	201.5 ± 7.0 e	205.5 ± 10 e	201.6 ± 6.8 e	197.5 ± 2.9 e	198.0 ± 5.5 e	197.2 ± 8.7 e	203.0 ± 2.2 e	198.7 ± 5.3	198.9 ± 6.9 e
T5-Untreated control	279.5 ± 23.0 a	288.0 ± 12.8 a	283.0 ± 14.3 a	297.7 ± 14 a	278.5 ± 7.0 a	285.3 ± 8.1 a	284.2 ± 21.7 a	285.2 ± 13.5 a	284.0 ± 15.7 a	289.0 ± 13.9 a	285.0 ± 21.0 a	285.5 ± 7.3 a
Mean	236.3 ± 5.6	235.3 ± 7.5	233.4 ± 8.4	237.9 ± 8.4	235.6 ± 7.3	–	233.2 ± 6.8	232.6 ± 7.2	233.9 ± 4.3	238.1 ± 5.4	235.0 ± 5.3	–
Sources of variation	d.f.		F value	Pr > F	SEd±		d.f.	F value	Pr > F	SEd±		
Varieties	4		0.56	0.37	3.08		4	1.07	0.37	2.95		
Soil treatments	4		223.32	<0.0001	3.08		4	254.64	<0.0001	2.95		
Interaction effect (varieties × Soil treatments)	16		0.87	0.99	6.90		16	0.15	0.99	6.60		
Error	72						72					

In a column, means followed by the same letter do not differ significantly from each other by Tukey's HSD ($\alpha = 0.05$).

[1]Each value is a mean of length of incisor cusps from four replications of a variety; n = 15.

Table 5 Effect of soil treatments on silicon content (mg/g) of rice stem tissue as influenced by varieties

	Wet season, 2011				Dry season, 2011–2012			
	Mean silicon content (mg/g)[1]				Mean silicon content (mg/g)[1]			
Varieties	1 DBA	7 DAA	7 DAB	Mean	1 DBA	7 DAA	7 DAB	Mean
BPT 5204	28.7 c	54.7 c	58.7 c	47.3	27. 7 c	55.4 c	59.0 c	47.3
MTU 1010	32.7 b	57.5 b	62.6 b	50.9	32.0 b	56.3 b	63.5 b	50.9
KRH 2	34.3 a	61.5 a	67.5 a	54.4	35.1 a	63.3 a	69.3 a	55.9
PB 1	26.4 d	51.9 d	55.4 d	44.5	27.1 d	52.4 d	54.2 d	44.5
Vandana	27.9 c	54.7 c	55.7 d	46.1	28.1 c	55. 7 c	57.6 c	47.1
d.f. (varieties, error)	4, 72	4, 72	4, 72	–	4, 72	4, 72	4, 72	–
F value	242.65	62.86	147.92	–	242.65	62.86	147.92	–
Pr > F	<0.0001	<0.0001	<0.0001	–	<0.0001	<0.0001	<0.0001	–
SEd±	0.30	0.65	0.59	–	0.40	0.72	0.63	–
Soil treatments								
T1-Rice husk ash (RHA)	30.1	49.7 d	55.3 c	45.0	31.3	51.2 d	58.0 d	46.8
T2-Imidazole	30.0	57.9 c	56. 9 c	48.3	30.3	61.2 c	63.3 c	51.6
T3-RHA+ imidazole (once)	30.0	66.9 b	71.8 b	56.2	31.3	72.1	76.2 b	59.8
T4-RHA+ imidazole (twice)	30.1	76.2 a	85.8 a	64.0	30.2	77.3 a	86.1 a	64.5
T5-Untreated control	29.7	29.7 e	30.0 d	29.8	28.7	30.2 e	31.0 e	29.9
d.f. (Soil treatments, error)	4, 72	4, 72	4, 72	–	4, 72	4, 72	4, 72	–
F value	0.49	1501. 66	2444.31	–	0.51	1604.14	2416.17	–
Pr > F	0.74	<0.0001	<0.0001	–	0.89	<0.0001	<0.0001	–
SEd±	0.30	0.65	0.59		0.40	0.72	0.63	–
Interaction effects (varieties × soil treatments)								
d.f. (varieties × Soil treatments, Error)	16, 72	16, 72	16, 72	–	16, 72	16, 72	16, 72	–
F value	0.78	2.66	10.18	–	0.83	3.12	12.04	–
Pr > F	0.70	0.25	0.34	–	0.69	0.0025	0.12	–
SEd±	0.66	1.14	1.04	–	0.95	1.62	1.42	–

DBA, day before application; DAA, days after application; DAB, days after booting stage.

[1]Mean values of silicon content from four replications. In columns, means followed by the same letter do not differ significantly from each other by Tukey's HSD ($\alpha = 0.05$).

T2-imidazole alone (11.1 ± 0.13%) and T1-RHA treatment (7.4 ± 0.05%) as compared to 3.6 ± 0.12% in T5-untreated control indicating 2.1- to 5.3-fold increase across the soil treatments.

Discussion

Silicon is a major biophysical factor that has a predominant negative impact on herbivores (Ma 2004). There is considerable evidence indicating the presence of a high quantity of silicon in rice cultivars imparting resistance or tolerance to various biotic and abiotic stresses (Savant et al. 1999; Ma 2004). Silicon nutrition in rice has been associated with the improved resistance to insect pests: African striped borer, *Chilo zacconius* Bleszynski (Lepidoptera: Pyralidae) (Ukwungwu 1984); yellow rice borer, *S. incertulas* (Panda et al. 1975); striped stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) (Sasamoto 1958); brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) (Yoshihara et al. 1979). However, the mechanisms involved in antiherbivory of Si are poorly understood or unexplored (Kvedaras et al. 2007).

In bio-efficacy studies, we reported variation among the selected rice cultivars in their native Si content as well as uptake of Si from soil solution as evidenced by biochemical estimation of Si, before and after application of treatments. Hou and Han (2010) showed genetic differences in silicon uptake ability among rice cultivars. In our experiment, the varieties did not have any resistant or tolerant factors which could affect the growth and development of YSB. But PB 1 was observed to be highly susceptible to YSB (Padmakumari and Pasalu 2003), followed by Vandana. In our study, T4-imidazole (twice) in combination with RHA proved to be the most effective treatment to reduce per cent damage in terms of both dead hearts and white ears followed by T3-RHA+ imidazole (applied once), T2-imidazole and T1-RHA. Djamin and Pathak (1967) reported that higher Si content in rice plant imparted resistance against *C. suppressalis*. There was no seasonal difference in the reaction of treated

plants against YSB. The present studies were in line with the findings of Ranganathan et al. (2006), Usha Rani et al. (2006) and Chandramani et al. (2010), who also tested some of the organic Si sources against YSB. Voleti et al. (2008) reported on the use of silicon solubilizers such as imidazole and some of the organic amino acids which reduced YSB damage in rice varieties.

In the present study, we clearly demonstrated the enhanced deposition of Si in stem tissues of rice plants upon soil application of RHA and imidazole either alone or in combination through SEMs, EDS and biochemical estimation of Si from stem tissues of rice plants. Maximum Si deposition was observed to an extent of 7.4–19.0% in treatments, although the rate of Si deposition varied across the soil treatments (Figure S5) as compared to the untreated control (3.6%). Many authors have reported different Si sources such as calcium silicate and calcium metasilicate (Anderson and Sosa 2001; Keeping and Meyer 2002; Correa et al. 2005; Goussain et al. 2005; Kvedaras et al. 2007), Na_2SiO_3 (Basagli et al. 2003) and K_2SiO_3 (Subbarao and Perraju 1976; Parrella et al. 2007). These chemicals are inorganic in nature, and their potential impact on actual physiology of rice crop is poorly understood. The use of bio-compatible organic molecules such as imidazole at low doses as a Si solubilizer and carrier benefits the crop in many ways without altering the plant physiology (Voleti et al. 2009). Many reports indicate that varieties differ in their Si uptake and accumulation capacity, and the Si deposition may vary from leaf to stem (Ma 2004; Voleti et al. 2008). Ranganathan et al. (2006) reported that silicon enhancement in the leaves of rice varieties increased by 3- to 5-fold due to the application of Si solubilizers. In a similar type of study, Voleti et al. (2008) reported 18–45% increase in Si content in the leaves which was confirmed through SEM and silicon mapping studies. In our experiment, a 2.1- to 5.3-fold increase was observed in Si content in the stem tissues across the soil treatments.

Variation in larval recovery (data not shown) and mortality from the treated stems could be attributed to the increase in Si content in the stem tissues in each of these treatments, which could have prevented the larvae from entering into the stem. With respect to per cent larval mortality and lowest larval weight, T4-RHA+ imidazole (twice) was the most effective followed by T3-RHA+ imidazole (once). However, mortality of the larvae was not observed in the treatment where T1-RHA was applied alone. In all the other parameters, RHA treatment was similar to imidazole treatment. The highest larval mortality in Si-treated plants confirms the findings of Subbarao and Perraju (1976) for YSB and Nozato (1982) for *C. suppressalis*.

In the current study, we reported maximum mandibular wear in YSB larvae collected from the Si-treated stems as measured in terms of mean length of incisor cusps. The least length of incisor cusps was noticed in T4-RHA+ imidazole (twice) followed by other treatments when compared to untreated control. This indicates that within an instar, feeding by larvae on the silicon-treated stems eroded their mandibles as there was silicon deposit in the stem tissues as observed in our SEM and EDS studies, resulting in low larval weight and high mortality. Defacing of larval mandibles due to feeding on rice cultivars having high Si content was reported for *C. suppresalis* by Sasamoto (1958); Djamin and Pathak (1967); Hou and Han (2010) and Alegre et al. (2011) in white stem borer, *Scirpophaga innotata* (Walker) (Lepidpoptera: Crambidae). However, in our studies we did not observe any significant differences in the length of larval incisors among the varieties tested as they were all susceptible to YSB. Chandramani et al. (2010) attributed the death of larval instars of *S. incertulas* to wearing of larval mandibles, leading to starvation upon fly ash application to the plants. However, Kvedaras et al. (2009) observed that although there was a trend for increased wear in larvae that developed on Si+ cane, no significant effect of silicon, cultivar or site on mandibular wear of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in sugarcane was shown.

This is the first report of its kind where an attempt was made to show the histological effect of Si on the gut lumen of larvae, as many authors have reported the other effects of silicon, such as feeding barrier, and biophysical basis of resistance on a wide range of insect pests (Djamin and Pathak 1967; Massey and Hartley 2006; Kvedaras et al. 2007). In the present study, we illustrate the effect of Si on histology of midgut epithelium through microtomy studies, where we observed completely ruptured peritrophic membrane, high vacuolation, distended and ruptured columnar cells and release of cell granules into the lumen due to abrasion of midgut epithelium of larvae collected from Si-treated plants. The compactness between columnar cells was lost completely because of damage to connective tissue. This could explain the lowest injury by YSB in rice plants with high Si content, highest larval mortality and lowest larval weight in soil treatments with Si when compared to untreated control. Similarly, leaf-folder *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae) larvae that fed on leaves where calcium silicate was applied had lower larval survival and pupation rates (Han et al. 2015).

The lower larval weight might be due to insufficient food intake due to mandibular wearing in the process of feeding or boring on Si-treated rice plants, and higher larval mortality might be due to starvation, due to worn out mandibles. Earlier studies clearly demonstrated that Si-rich plants have a significant negative impact on herbivore performance, digestibility, growth and development (Goussain et al. 2005; Kvedaras et al. 2007; Alegre et al. 2011). Changes in the alimentary canal, especially in the region of the midgut, can affect the growth and development of insects, because these processes depend on enzyme secretion, adequate food intake and its absorption and transformation in the alimentary canal (Mordue (Luntz) and Nisbet 2000). In our experiment, the weight of larvae recovered from silicon-treated plants was significantly less, and mortality rate was very high compared to the control. This might be because of the mechanical injury to midgut epithelium due to abrasion by Si as evidenced in our microtomy study and further inability of the larvae to feed, digest and absorb.

We conclude that application of a renewable source of Si (RHA) and carrier-induced Si (imidazole), alone or in combination, at two vulnerable stages of rice crop growth reduced the damage by YSB, a serious pest of rice. This is the first study where an effect of Si on larval growth, larval recovery, mandible wearing and inner lining of the midgut of YSB was demonstrated. The mode of action can be summarized as an application of Si (imidazole/RHA) to the soil reduced the injury caused by the pest to rice plant at all stages of growth. This is supported by our EDS studies in which we showed that there was an increase in Si content (2.1- to 5.3-fold) in the stem tissues (which is a port of entry for YSB larva) in the treatments as compared to the untreated control. Our studies also indicated lower borer damage in Si-treated plants which could be due to wearing of larval incisors. High larval mortality was observed in imidazole treatments. Disruption of the midgut lining as evidenced from microtomy studies shows that Si can also adversely affect midgut because of abrasion, thereby causing physiological effects. The role of Si in alleviating abiotic and biotic stresses and its effect on plant growth are more pronounced in stress conditions (Tamai and Ma 2008; Meharg and Meharg 2015). Application of Si in crops provides a viable component of integrated management of pests and diseases because it leaves no pesticide residues in food or the environment, and it can be easily integrated with other management practices including biological control (Laing et al. 2006). RHA is an agricultural waste obtained by rice milling and includes a large amount of Si with high specific surface that is very suitable for soil stabilization and is economical particularly in high-production regions. Thus, our study shows that application of cost-effective and naturally available Si sources or materials that make Si available at very low doses can be chosen as a viable component in the management of rice pests in general and YSB in particular.

Author Contributions

APP and TUM conceived and designed research. MJ conducted experiments and analysed data. MJ and APP wrote the manuscript. APP provided the logistic support. SRV provided useful information for carrying out experiments. All authors read and approved the manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mandible of a YSB larva. L1-Width of the mandible; L2 = length from the base of the mandible to tip of the incisor region.

Figure S2. (a) Dead heart. (b) White ear.

Figure S3. Histological effects of soil treatments with Si on midgut of YSB larvae collected from variety BPT 5204.

Figure S4. Scanning electron micrographs of rice stem tissue from different soil treatments in variety BPT 5204.

Figure S5. Electron dispersive X-ray spectrum (EDS) of rice stem tissues in variety BPT 5204 showing the elemental Si accumulation in various soil treatments (*X*-axis-keV, *Y* axis-counts).

Table S1. Treatment details.

Table S2. Effect of soil treatments on per cent damage by YSB as influenced by varieties in greenhouse experiments, dry season, 2011–2012.