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ORIGINAL PAPER

Different Silicon Application Sources and Factors Influencing Mandibular Wear in Sugarcane Early Shoot Borer, *Chilo infuscatellus* Walker

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Abstract
Silicon (Si) can improve plant resistance to herbivorous insect attack. Early shoot borer (ESB), is one of the most solemn lepidopterans borers on sugarcane in India. Even with insecticide application, management of this pest is difficult. The management requires a fresh new approach. As a result, the current study aims to determine the effect of silicon on the early shoot borer, *Chilo infuscatellus* Walker, through biophysical factor analysis and mandibular wear documentation. When sugarcane leaves were treated with calcium silicate at 1000 kg/ha, scanning electron microscopy revealed the presence of silicified cells and trichomes, as well as increased cell wall thickness, silicon content, and waxes content. Furthermore, larvae of an early shoot borer fed silica-treated plants showed a complete wearing of mandibles as compared to untreated check.

Keywords *Chilo infuscatellus* · Mandibular wear · Silicon · Silicified cells and sugarcane

1 Introduction

Sugarcane, *Saccharum officinarum* L., is one of the world's most important cash crops grown in tropical and subtropical countries, as well as in many developing countries; it is a major source of foreign exchange. India is the world's second largest producer of sugarcane, trailing only Brazil. It is cultivated in approximately 26 million hectares with a worldwide production of 1.89 billion tonnes, and in India, it is cultivated in approximately 4.927 million hectares of land with an annual production of 348 million tonnes and productivity of 70.7 tonnes per hectare during 2015–16, whereas in Tamil Nadu, it is cultivated in approximately 2.18 lakh hectares with a production of 189.88 lakh tonnes and productivity of 87.1 tonnes per hectares [1].

In sugarcane, lepidopteran pest is the most serious cause for yield loss. Early shoot borer (ESB), *Chilo infuscatellus* Walker is a destructive pest among lepidopterans. The larvae of borer can cause dead heart in vegetative stage which results in reduction in cane yield as well as sugar recovery. In the sugarcane ecosystem, pest management with insecticides and bioagents is complicated. An alternative new approach to managing the early shoot borer is the use of silicon fertilizers. Silicon (Si) is generally not considered an essential element but it is an important nutrient that promotes growth and development of many agricultural and horticultural crops [2]. Silicon is the only known nutrient that is not detrimental when accumulated in excess [3]. Silicon amendments enhance resistance to attack by herbivorous insects including borers [4]. Silicon helps in strengthening the cell walls and thereby making the plants hard and stiff. Hence, lepidopteran borers find difficulty to feed and their mandibles get broken. Sugarcane accumulates the most silicon among agricultural crops and can use it efficiently in plant growth and insect resistance [5, 6]. Reduced digestibility and increased hardness and abrasiveness of plant tissues due to silica deposition in various tissues such as epidermal silica cells [7] and the compositions of the silica content in calcium silicate and rice husk ash was 20.1% and 34.7% [8] are the mechanisms for silicon's action in increasing plant

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resistance to insect attack. Based on mandibular wear of larvae, current research was focused on the association between silicon incorporation and host-plant resistance to the early shoot borer, *Chilo infuscatellus.*

2 Material and Methods

2.1 Location and Experimental Details

Two field experiments were carried out, one at a farmer's holding in Poovanthi (February 2017 - January 2018) and the other at Silaiman (May 2017 - February 2018) village in the Sivagangai and Madurai districts, respectively, to assess the effect of silicon by basal application of silicon sources such as rice husk ash, baggase ash, calcium silicate, and sodium metasilicate on sugarcane early shoot borer. The design used was a Randomized block design (RBD) with the variety CO Si 2016 and the following nine treatments, which was replicated three times. T_1 - Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}, T_2 - Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}, T_3 - Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}, T_4 - Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}, T_5 - Calcium silicate @ 500 kg ha^{-1}, T_6 - Calcium silicate @ 1000 kg ha^{-1}, T_7 - Sodium meta silicate @ 500 kg ha^{-1}, T_8 - Sodium meta silicate @ 1000 kg ha^{-1} and T_9 - Untreated check. The plot size was 6×3 m^2 and all the agronomic practices were followed uniformly in all the plots. For documentation of mandibular wear, the larvae of early shoot borer were collected from selected treated and untreated plots after 60 DAP. Laboratory experiments were conducted by collecting the leaf samples from the field during critical phases of the crop viz*.,* 90, 180 and 270 DAT in all treated plots and utilized for analyzing certain bio physical factors.

2.2 Silicified Cells

Fresh leaf samples were soaked in 70 percent ethanol for 3 days. Selected tissue in which silicified cells are to be examined were cut into 1 sq cm long pieces. The sample pieces were kept in a beaker containing 20 ml of the safranin-phenol reagent and boiled gently for 1 min. The sample pieces were removed from the beaker and placed on a glass slide with 2 drops of hot safranin-phenol reagent. The leaf tissue was covered with a cover glass and kept under microscope. The optimal magnification usually ranged from 50 × to 100x. The silicified cells were observed as dumbbell shaped clear to grey areas in the leaf tissue [9]. Scanning electron microscopy (SEM) was used to examine the ultrastructure of silicified cells at an accelerating voltage of 25 keV and a magnification of 2000 × for morphological observation [10].

2.3 Trichome Density

Sugarcane leaf samples were randomly collected from the experimental plots, cut into 1 sq cm bits, and boiled for 45 min in small glass vials in a hot water bath at 85 °C. The water was then poured out, leaving the leaves behind, which were then boiled in 96% ethanol at 80 °C for 30 min. The alcohol was then poured out. To completely remove the chlorophyll from the leaves, the boiling process with alcohol was repeated. After removing the alcohol, 90% lactic acid was added and heated at 85 °C until the leaf bits were completely clear. The vials were then cooled, and leaf fragments were taken and mounted on clean glass slides with a drop of lactic acid to estimate the trichome count. The number of trichomes per square centimeter was counted under compound microscope (45 × magnifications) for each sample [11].

2.4 Wax Content

Sugarcane leaves of known length (1 cm^2) were collected from all test plots and immersed in chloroform (approximately 25–30 ml in a beaker) for 10 min. At room temperature, the extracts were allowed to evaporate. After evaporation, the weight of the condensed wax in and around the bottom of the beaker was measured, and the experiment was repeated three times [12].

2.5 Silicon Content

Each treatment's leaf sample was dried in an oven at 70–80 °C and ground into a fine powder. 1 g of each sample was weighed and placed in a 100 ml conical flask with 15 ml of triacid (conc.HNO3: Sulphuric acid: Perchloric acid in a 9:2:1 ratio) and shaken for a few seconds to wet the powder. The conical flask's contents were then heated on the electrical heater to reduce heat intensity. When white fumes followed brown fumes, the flasks were removed from the heater. This meant that the digestion was finished. After cooling to room temperature, it was diluted with distilled water and filtered through pre-weighed Whatman 42 filter paper. This silicon residue (SiO2), left over the filter paper was washed several times with distilled water and dried in an electric oven at 50–55 °C. After complete drying, it was weighed on an electric balance. The weight of dried silica was determined by the difference between the weight of the filter paper and the total weight. The amount of silica in one gram dry weight of leaf was converted into percentage [13].

2.6 Cell Wall Thickness

Sugarcane leaves from silicon-treated plants were fixed in FAA70 (formaldehyde, acetic acid, ethanol 50%, 5:5:90, v/v) for 48 h and preserved in 70% ethanol. 5 mm^2 leaf blade samples were dehydrated in an ethylic series (70 to 95%) and embedded in a histological resin (Historesin Leica). The samples were kept in a dehydrator under constant vacuum to facilitate solution penetration. The samples were sectioned in transversal and longitudinal planes using a glass razor and an automatic advancement rotary microtome. Cuts 5 to 7 µm thick were distended for 10 min on glass plates stained with Blue Toluidine in pH 4.4 and mounted with a synthetic resin. The sections were digitalized using a compound microscope attached to a digital camera and connected to a microcomputer [14].

2.7 Mandibular Wear of Early Shoot Borer

Ten third instar larvae of the early shoot borer, *Chilo infuscatellus*, were collected from sugarcane treated plots with silicon fertilizers from farmer's holdings in the villages of Poovanthi and Silaiman in the districts of Sivagangai and Madurai, respectively. Larvae were kept in 70% alcohol until they were needed. The larvae were removed from the alcohol and allowed to air dry for 10 min before being examined under a light microscope. The labrum was removed with a fine-tipped dissecting needle, and the mandibles were carefully dissected. Under a compound microscope, the dissected larval mandibles were examined for mandibular wear [15]. Early shoot borer mandibular wear was measured from digital images using Axio lab solutions under a compound microscope.

2.8 Statistical Analysis

Data collected in the field, pot culture, and laboratory experiments was statistically analyzed. An angular transformation was applied to the percentage values. The population numbers were converted using the square root transformation. For significance, the treatment means were compared using the least significant difference (LSD) [16].

3 Results and Discussion

Pooled analysis and correlation of physical factors of two field experiments data on basal application of silicon sources is presented in Tables 1, 2, 3, 4 and 5; Figs.5 and 6.

3.1 Silicified cells

The number of silicified cells deposition differed significantly between treatments in the first field experiment at Poovanthi, Sivagangai district, at 90, 180, and 270 DAP (Table 1). The mean number of silicified cells overall varied from 7.84 to 18.68/cm2 among treatments, compared to 4.73/cm2 in the untreated check (Fig. 1). At 90 DAP, the number of silicified cells was significantly higher in plants treated with calcium silicate 1000 kg/ha (15.66 nos./cm2) than in plants treated with baggase ash 1000 kg/ha + SSB 2 kg/ha (13.20 nos./cm2). The next best treatments were baggase ash at 500 kg/ha + SSB at 2 kg/ha and calcium silicate at 500 kg/ha (11.44 and 11.27 nos./cm2, respectively). The silicified cells recorded for sodium metasilicate at 1000 kg/ha were 9.08 nos./cm2, followed by sodium metasilicate at 500 kg/ha and rice husk ash at 1000 kg/ha + SSB @2 kg/ha (8.32 and 8.13 nos./cm2). The silicified cells recorded in rice husk ash at 500 kg/ha were less (5.43 nos./cm2) than in the untreated control (3.73 nos./cm2). In terms of silicified cells, the 180 DAP trend was similar to the 90 DAP trend. At 270 DAP, the number of silicified cells in calcium silicate and calcium silicate at 1000 and 500 kg/ha (21.64 and 21.29 nos./cm2) was significantly higher.

In the second field experiment at Silaiman, Madurai district, the number of silicified cells differed significantly among various treatments at 90, 180 and 270 DAP. The overall mean silicified cells ranged from 19.30 to 10.26 nos./cm^2 among the treatments as against 5.04 nos./cm^2 in untreated check. At 90 DAP, silicified cells was significantly high in calcium silicate 1000 kg/ha treated plants (14.63 nos./cm^2) followed by baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (14.36 nos./cm^2) and the number of silicified cells was significantly less in rice husk ash at 500 kg/ha + SSB @ 2 kg/ha (7.45 nos./cm^2). At 180 DAP, the number of silicified cells was significantly high in both calcium silicate 1000 and 500 kg/ha treated plants (19.58 and 19.00 nos./cm^2). Whereas at 270 DAP, the number of silicified cells was significantly high in calcium silicate at 500 kg/ha (25.26 nos./cm^2) followed by baggase ash @ 500 kg/ha + SSB @ 2 kg/ha and calcium silicate at 1000 kg/ha (23.88 and 23.70 nos./cm^2). The present study is in line with the findings of [17] reported that the presence of silicified cells was significantly high in sodium metasilicate 2t/ha treated plants (27.30 nos./cm^2) followed by calcium silicate at 2t/ha (25.60 nos./cm^2) and showed negative effect on population of leaf folder and spiny beetle.

3.2 Trichome Density

In the first field experiment at Poovanthi, Sivagangai district, the trichome density differed significantly among various treatments at 90, 180 and 270 DAP. The overall

Table 1 Estimation of silicified cells in sugarcane leaves as influenced by silicon sources

S. No	Treatments	Field experiment I			Mean	Field experiment II			Mean
		Silicified cells (No./cm^2)*				Silicified cells (No./cm^2)*			
		90 DAP	180 DAP	270 DAP		90 DAP	180 DAP	270 DAP	
T_1	Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	5.43 (2.33)^f	8.63 (2.94)^g	9.80 (3.13)^f	7.84 (2.82)^c	7.45 (2.73)^f	9.41 (3.07)^f	13.92 (3.73)^f	10.26 (3.20)^b
T_2	Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	8.13 (2.85)^e	9.76 (3.12)^f	12.40 (3.52)^e	10.10 (3.18)^c	8.54 (2.92)^e	10.98 (3.31)^e	13.62 (3.69)^f	11.05 (3.32)^b
T_3	Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	11.44 (3.38)^c	13.85 (3.72)^d	18.86 (4.34)^b	14.72 (3.84)^{ab}	13.65 (3.69)^b	19.67 (4.43)^a	23.88 (4.89)^b	19.06 (4.37)^a
T_4	Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	13.20 (3.63)^b	16.89 (4.11)^b	18.63 (4.32)^b	16.24 (4.03)^{ab}	14.36 (3.79)^{ab}	17.28 (4.16)^b	21.93 (4.69)^c	17.86 (4.23)^a
T_5	Calcium silicate @ 500 kg ha^{-1}	11.27 (3.36)^c	15.66 (3.96)^c	21.29 (4.61)^a	16.08 (4.01)^{ab}	12.53 (3.54)^c	19.00 (4.36)^a	25.26 (5.03)^a	18.93 (4.35)^a
T_6	Calcium silicate @ 1000 kg ha^{-1}	15.66 (3.96)^a	18.75 (4.33)^a	21.64 (4.65)^a	18.68 (4.32)^a	14.63 (3.82)^a	19.58 (4.42)^a	23.70 (4.87)^b	19.30 (4.39)^a
T_7	Sodium meta silicate @ 500 kg ha^{-1}	8.32 (2.88)^e	11.29 (3.36)^e	14.06 (3.75)^d	11.22 (3.35)^{bc}	9.11 (3.02)^e	13.07 (3.61)^c	16.24 (4.03)^e	12.80 (3.58)^{ab}
T_8	Sodium meta silicate @ 1000 kg ha^{-1}	9.08 (3.01)^d	10.07 (3.17)^f	15.79 (3.97)^c	11.64 (3.41)^{bc}	9.87 (3.14)^d	12.24 (3.50)^d	17.37 (4.17)^d	13.16 (3.63)^{ab}
T_9	Untreated check	3.73 (1.93)^g	5.16 (2.27)^h	5.29 (2.30)^g	4.73 (2.17)^d	4.12 (2.03)^g	5.69 (2.39)^g	6.32 (2.51)^g	5.04 (2.25)^c
SEd		0.04	0.05	0.05	0.29	0.04	0.05	0.06	0.41
CD (P = 0.05)		0.10	0.11	0.11	0.62	0.10	0.11	0.12	0.87

*Mean of three replications

Figures in parentheses are square root transformed values

In a column means followed by the same letter(s) are not significantly different by LSD (p = 0.05)

DAP Days after planting, *SSB* Silica solubilizing bacteria

mean trichome density ranged from 11.34 to 25.72 nos./cm^2 among the treatments and in untreated check it was 5.85 nos./cm^2 (Fig. 2). At 90 DAP, the trichome density was significantly high in calcium silicate 1000 kg/ha treated plants (19.37 nos./cm^2) followed by calcium silicate @ 500 kg/ha (18.06 nos./cm^2) and in untreated check, it was 4.22 nos./cm^2. In rice husk ash at 500 kg/ha + SSB @ 2 kg/ha, the trichome density recorded was less (9.21 nos./cm^2). Similar trend was observed at 180 and 270 DAP (Fig. 5).

In the second field experiment at Silaiman, Madurai district, the overall mean trichome density ranged from 12.78 to 26.90 nos./cm^2 among the treatments as against 6.40 nos./cm^2 in untreated check. At 90 DAP, trichome density was significantly high in calcium silicate 1000 kg/ha treated plants (19.37 nos./cm^2) followed by baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (18.73 nos./cm^2) and calcium silicate at 500 kg/ha (18.48 nos./cm^2). As that of first experiment, the trichome density was less in rice husk ash at 500 kg/ha + SSB @ 2 kg/ha (10.20 nos./cm^2). Similar trend was also observed at 180 and 270 DAP (Table 2). The present study is in line with the findings of [18] reported that the applications of lignite fly ash @ 25 kg/ha increased the number of leaf trichomes and showed resistance to rice leaf folder. Similarly, [19] reported that application of silicon enriched the trichomes density which mechanically affects the insects, impeding their movement and settlement and possibly negatively affecting their oviposition preference and feeding rate. Marafon and Endres [20] reported that silicon improved the plant fitness mainly through the formation of structures such as trichomes, thorns and spines. Dorairaj and Ismail [21] reported that trichomes impart strength to the plant and serve as a mechanical barrier against stem borers and planthoppers in rice.

3.3 Wax Content

In field experiments conducted at Poovanthi, Sivagangai district and Silaiman, Madurai district, the leaf wax content gradually increased as the plants attained maturity in all the treatments (Table 3). In the first field experiment, the wax content differed significantly among various treatments at 90, 180 and 270 DAP. The overall mean wax content ranged from 0.229 to 0.330 mg/g among the treatments and in untreated check it was 0.170 mg/g. At 90 DAP, the wax content was significantly high in calcium silicate 1000 kg/ha treated plants.

Table 2 Estimation of trichomes density in sugarcane leaves as influenced by silicon sources

S. No	Treatments	Field experiment I			Mean	Field experiment II			Mean
		Number of trichomes/cm^{-2} *				Number of trichomes/cm^{-2} *			
		90 DAP	180 DAP	270 DAP		90 DAP	180 DAP	270 DAP	
T_1	Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	9.21 (3.04)^{g}	10.78 (3.28)^{f}	14.02 (3.74)^{f}	11.34 (3.37)^{c}	10.20 (3.19)^{e}	12.94 (3.60)^{e}	15.19 (3.90) ^{f}	12.78 (3.57)^{b}
T_2	Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	9.96 (3.16)^{f}	11.59 (3.40)^{e}	15.96 (4.00)^{e}	12.51 (3.54)^{c}	11.49 (3.39)^{d}	10.57 (3.25)^{f}	16.57 (4.07)^{e}	12.88 (3.59)^{b}
T_3	Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	15.35 (3.92)^{c}	19.87 (4.46)^{c}	26.29 (5.13)^{b}	20.50 (4.53)^{ab}	18.16 (4.26)^{b}	27.39 (5.23)^{ab}	31.61 (5.62)^{b}	25.72 (5.07)^{a}
T_4	Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	14.17 (3.76)^{d}	19.60 (4.43)^{c}	24.75 (4.97)^{c}	19.51 (4.42)^{a}	18.73 (4.33)^{ab}	25.72 (5.07)^{c}	29.70 (5.45)^{c}	24.72 (4.97)^{a}
T_5	Calcium silicate @ 500 kg ha^{-1}	18.06 (4.25)^{b}	24.84 (4.98)^{b}	30.48 (5.52)^{a}	24.46 (4.95)^{a}	18.48 (4.30)^{ab}	28.81 (5.37)^{a}	33.40 (5.78)^{a}	26.90 (5.19)^{a}
T_6	Calcium silicate @ 1000 kg ha^{-1}	19.37 (4.40)^{a}	26.17 (5.12)^{a}	31.63 (5.62)^{a}	25.72 (5.07)^{a}	19.37 (4.40)^{a}	26.89 (5.19)^{bc}	30.81 (5.55)^{bc}	25.69 (5.07)^{a}
T_7	Sodium meta silicate @ 500 kg ha^{-1}	10.20 (3.19)^{f}	12.08 (3.48)^{e}	16.14 (4.02)^{de}	12.80 (3.58)^{c}	10.40 (3.22)^{e}	12.18 (3.49)^{e}	16.04 (4.00)^{ef}	12.87 (3.59)^{b}
T_8	Sodium meta silicate @ 1000 kg ha^{-1}	11.35 (3.37)^{e}	12.93 (3.60)^{d}	17.17 (4.14)^{d}	13.82 (3.72)^{bc}	12.53 (3.54)^{c}	15.59 (3.95)^{d}	17.86 (4.23)^{d}	15.33 (3.92)^{b}
T_9	Untreated check	4.22 (2.05)^{h}	5.74 (2.40)^{g}	7.60 (2.76)^{g}	5.85 (2.42)^{d}	5.00 (2.24)^{f}	7.06 (2.66)^{g}	7.16 (2.68)^{g}	6.40 (2.53)^{c}
SEd		0.05	0.05	0.06	0.38	0.05	0.06	0.06	0.42
CD (P = 0.05)		0.10	0.11	0.13	0.81	0.11	0.13	0.14	0.88

*Mean of three replications

Figures in parentheses are square root transformed values

In a column means followed by the same letter(s) are not significantly different by LSD (p = 0.05)

DAP Days after planting, *SSB* Silica solubilizing bacteria

(0.218 mg/g) followed by calcium silicate at 500 kg/ha (0.207 mg/g) and in untreated check, it was 0.105 mg/g. In rice husk ash at 500 kg/ha + SSB @ 2 kg/ha, the wax content recorded was less (0.139 mg/g). Similar trend was observed at 180 DAP. Whereas at 270 DAP, the wax content was significantly high in baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (0.471 mg/g) followed by calcium silicate at 500 kg/ha (0.438 mg/g) and in untreated check, it was 0.214 mg/g.

In the second field experiment, the overall mean wax content ranged from 0.297 to 0.388 mg/g among the treatments as against 0.230 mg/g in untreated check. At 90 DAP, wax content was significantly high in calcium silicate 1000 kg/ha treated plants (0.266 mg/g) followed by baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (0.248 mg/g), baggase ash at 500 kg/ha + SSB @ 2 kg/ha (0.246 mg/g) and calcium silicate at 500 kg/ha (0.244 mg/g) and these three treatments were found to be on par with each other. As that of first experiment, wax content was recorded less in rice husk ash at 500 kg/ha (0.202 mg/g). At 180 DAP, the wax content was significantly high in calcium silicate at 500 and 1000 kg/ha (0.386 and 0.386 mg/g) followed by baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (0.361 mg/g) and in untreated check, it was 0.267 mg/g. Similar trend was also observed at 270 DAP. The present study is in line with the findings of [22] (reported that 1 g of calcium silicate enhanced the resistance to insect feeding through the formation of a thicker wax layer on the abaxial surface of coffee seedlings.

3.4 Silica Content

In field experiments conducted at Poovanthi, Sivagangai district and Silaiman, Madurai district, the silica per cent gradually increased from 90 DAP onwards and reached a peak at 270 DAP (Table 4). In the first field experiment, the silica per cent differed significantly among various treatments at 90, 180 and 270 DAP. The overall mean ranged from 1.01 to 1.58 per cent among the treatments and in untreated check it was 0.50%. At 90 DAP, the silica per cent was significantly high in calcium silicate at 500 and 1000 kg/ha (1.36 and 1.36%) followed by baggase ash at 500 kg/ha + SSB @ 2 kg/ha (1.26%) and in untreated check, it was 0.31 per cent. Among the treatments, less silica per cent was recorded in rice husk ash at 500 kg/ha + SSB @ 2 kg/ha (0.86%). As that of 90 DAP, similar trend was also observed at 180 and 270 DAP.

Table 3 Estimation of wax content in sugarcane leaves as influenced by various silicon sources

S. No	Treatments	Field experiment I			Mean	Field experiment II			Mean
		Wax content (mg/g)*				Wax content (mg/g)*			
		90 DAP	180 DAP	270 DAP		90 DAP	180 DAP	270 DAP	
T_1	Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.139 $(0.373)^{e}$	0.240 $(0.490)^{e}$	0.308 $(0.555)^{d}$	0.229 $(0.479)^{b}$	0.202 $(0.449)^{d}$	0.311 $(0.557)^{cd}$	0.410 $(0.640)^{cd}$	0.307 $(0.555)^{b}$
T_2	Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.157 $(0.396)^{d}$	0.260 $(0.510)^{d}$	0.315 $(0.561)^{cd}$	0.244 $(0.494)^{b}$	0.216 $(0.464)^{c}$	0.329 $(0.574)^{c}$	0.417 $(0.646)^{c}$	0.321 $(0.566)^{b}$
T_3	Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.180 $(0.424)^{c}$	0.299 $(0.547)^{c}$	0.418 $(0.647)^{b}$	0.299 $(0.547)^{a}$	0.246 $(0.496)^{b}$	0.368 $(0.607)^{ab}$	0.514 $(0.717)^{a}$	0.376 $(0.613)^{a}$
T_4	Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.175 $(0.418)^{c}$	0.273 $(0.522)^{d}$	0.471 $(0.686)^{a}$	0.306 $(0.553)^{a}$	0.248 $(0.498)^{b}$	0.361 $(0.601)^{b}$	0.478 $(0.692)^{b}$	0.363 $(0.602)^{a}$
T_5	Calcium silicate @ 500 kg ha^{-1}	0.207 $(0.455)^{b}$	0.326 $(0.571)^{b}$	0.438 $(0.662)^{b}$	0.324 $(0.569)^{a}$	0.244 $(0.494)^{b}$	0.386 $(0.621)^{a}$	0.529 $(0.727)^{a}$	0.387 $(0.622)^{a}$
T_6	Calcium silicate @ 1000 kg ha^{-1}	0.218 $(0.467)^{a}$	0.346 $(0.588)^{a}$	0.427 $(0.653)^{b}$	0.330 $(0.575)^{a}$	0.266 $(0.516)^{a}$	0.386 $(0.622)^{a}$	0.513 $(0.716)^{a}$	0.388 $(0.623)^{a}$
T_7	Sodium meta silicate @ 500 kg ha^{-1}	0.147 $(0.383)^{e}$	0.269 $(0.519)^{d}$	0.315 $(0.561)^{cd}$	0.244 $(0.493)^{b}$	0.218 $(0.467)^{c}$	0.311 $(0.558)^{cd}$	0.388 $(0.623)^{de}$	0.306 $(0.553)^{b}$
T_8	Sodium meta silicate @ 1000 kg ha^{-1}	0.148 $(0.385)^{de}$	0.261 $(0.510)^{d}$	0.332 $(0.576)^{c}$	0.247 $(0.497)^{b}$	0.207 $(0.455)^{cd}$	0.304 $(0.551)^{d}$	0.379 $(0.616)^{e}$	0.297 $(0.545)^{b}$
T_9	Untreated check	0.105 $(0.324)^{f}$	0.192 $(0.438)^{f}$	0.214 $(0.462)^{e}$	0.170 $(0.413)^{c}$	0.115 $(0.339)^{e}$	0.267 $(0.516)^{e}$	0.310 $(0.557)^{f}$	0.230 $(0.480)^{c}$
SEd		0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01
CD (P = 0.05)		0.01	0.01	0.01	0.03	0.01	0.01	0.02	0.03

*Mean of three replications

Figures in parentheses are square root transformed values

In a column means followed by the same letter(s) are not significantly different by LSD (p = 0.05)

DAP Days after planting, *SSB* Silica solubilizing bacteria

In the second field experiment, the overall mean ranged from 1.30 to 1.79 per cent among the treatments and in untreated check it was 0.55 per cent. At 90 DAP, the silica per cent was significantly high in calcium silicate at 1000 kg/ha (1.67%) followed by calcium silicate at 500 kg/ha and baggase ash at 500 kg/ha + SSB @ 2 kg/ha (1.57 and 1.53%) and in untreated check, it was 0.39 per cent. Among the treatments, less silica per cent was recorded in sodium metasilicate at 500 kg/ha + SSB @ 2 kg/ha (1.09%). At 180 DAP, the silica per cent was significantly high in calcium silicate at 1000 and 500 kg/ha (1.77 and 1.75%) and baggase ash at 500 kg/ha + SSB @ 2 kg/ha (1.71%) followed by baggase ash at 1000 kg/ha + SSB @ 2 kg/ha (1.51%) and in untreated check, it was 0.55%. At 270 DAP, the silica per cent was significantly high in calcium silicate at 500 kg/ha and baggase ash at 500 kg/ha + SSB @ 2 kg/ha (2.05 and 1.99%) followed by calcium silicate at 1000 kg/ha (1.85%) and in untreated check, it was 0.71 per cent.

The present study is in line with the findings of [23] who suggested that at least 1% Si is required for optimal cane yield and at 0.25% Si, the yield drops to about 50% in Florida. Korndorfer et al. [24] further reported that sugarcane variety cv. SP79-1011 with highest Si content in leaves (2.31% Si) and the two lower Si-accumulating sugarcane varieties cv. SP80-1816 and SP81-3250 having 1.60% and 1.56% Si respectively due to silicon fertilization. The present study is again in agreement with the findings of [25] who reported that the rice stem attacked by the stem borer was found to contain a lower amount of Si. Hosseini et al. [26] reported that the silica content in rice stem was increased by 7.56, 9.53 and 30.81 per cent at SiO_2 fertilization levels of 5, 10 and 20 g compared to control respectively. Arivuselvi et al. [17] who reported that the rice plants treated with sodium metasilicate at 2t/ha recorded maximum silica content (9.88%) followed by calcium silicate at 2t/ha treated plants (9.03%) resulted in the reduced the incidence of brown plant hopper. Parthiban et al. [27] who reported that the foliar spray of calcium silicate at 5.0% reduced the population of leaf miner in groundnut was due to high accumulation of silica in groundnut plants.

3.5 Cell Wall Thickness

In the present study, the results revealed clearly the cell wall thickness under phrase contrast microscope was 0.020 and

Table 4 Estimation of silica content in sugarcane leaves as influenced by different sources of silicon

Treatments	Field experiment I			Mean	Field experiment II			Mean
	Silicon content (%)				Silicon content (%)			
	90 DAP	180 DAP	270 DAP		90 DAP	180 DAP	270 DAP	
T_1 - Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.86 (5.33)^{e}	0.98 (5.68)^{f}	1.18 (6.23)^{d}	1.01 (5.76)^{e}	1.10 (6.01)^{e}	1.29 (6.53)^{d}	1.51 (7.06)^{d}	1.30 (6.55)^{d}
T_2 - Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	1.00 (5.73)^{d}	1.20 (6.29)^{d}	1.32 (6.60)^{c}	1.17 (6.22)^{d}	1.22 (6.34)^{d}	1.42 (6.85)^{c}	1.63 (7.33)^{c}	1.42 (6.85)^{c}
T_3 - Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	1.26 (6.46)^{b}	1.46 (6.95)^{b}	1.61 (7.28)^{b}	1.44 (6.90)^{b}	1.53 (7.09)^{b}	1.71 (7.50)^{a}	1.99 (8.10)^{a}	1.74 (7.58)^{b}
T_4 - Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	1.09 (5.98)^{c}	1.28 (6.50)^{c}	1.59 (7.25)^{b}	1.32 (6.60)^{c}	1.32 (6.60)^{c}	1.51 (7.07)^{b}	1.65 (7.38)^{c}	1.49 (7.02)^{c}
T_5 - Calcium silicate @ 500 kg ha^{-1}	1.36 (6.69)^{a}	1.57 (7.19)^{a}	1.77 (7.66)^{a}	1.57 (7.19)^{a}	1.57 (7.19)^{b}	1.75 (7.61)^{a}	2.05 (8.22)^{a}	1.79 (7.69)^{a}
T_6 - Calcium silicate @ 1000 kg ha^{-1}	1.36 (6.70)^{a}	1.61 (7.28)^{a}	1.77 (7.65)^{a}	1.58 (7.22)^{a}	1.67 (7.42)^{a}	1.77 (7.65)^{a}	1.85 (7.83)^{b}	1.77 (7.64)^{a}
T_7 - Sodium meta silicate @ 500 kg ha^{-1}	0.99 (5.71)^{d}	1.08 (5.98)^{e}	1.39 (6.76)^{c}	1.15 (6.16)^{d}	1.09 (5.99)^{e}	1.37 (6.71)^{cd}	1.39 (6.76)^{e}	1.28 (6.50)^{d}
T_8 - Sodium meta silicate @ 1000 kg ha^{-1}	1.09 (5.98)^{c}	1.26 (6.45)^{cd}	1.60 (7.26)^{b}	1.31 (6.58)^{c}	1.24 (6.40)^{d}	1.44 (6.89)^{bc}	1.56 (7.17)^{cd}	1.41 (6.83)^{c}
T_9 - Untreated check	0.31 (3.21)^{f}	0.43 (3.77)^{g}	0.76 (3.77)^{e}	0.50 (4.07)^{f}	0.39 (3.59)^{f}	0.55 (4.25)^{e}	0.71 (4.82)^{f}	0.55 (4.25)^{e}
SEd	0.08	0.09	0.10	0.09	0.09	0.09	0.10	0.09
CD (P = 0.05)	0.17	0.19	0.21	0.19	0.19	0.20	0.21	0.02

*Mean of three replications

Figures in parentheses are arc sine transformed values

In a column means followed by the same letter(s) are not significantly different by LSD (p = 0.05)

DAP Days after planting, *SSB* Silica solubilizing bacteria

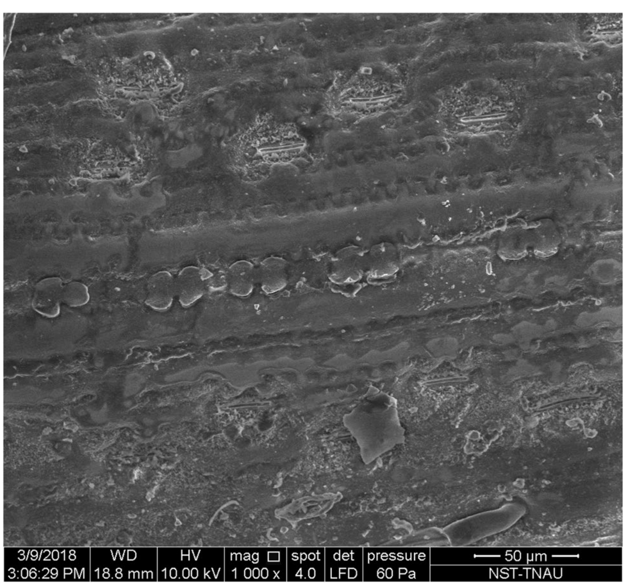

Fig. 1 Sugarcane silicified cells under scanning electron microscope

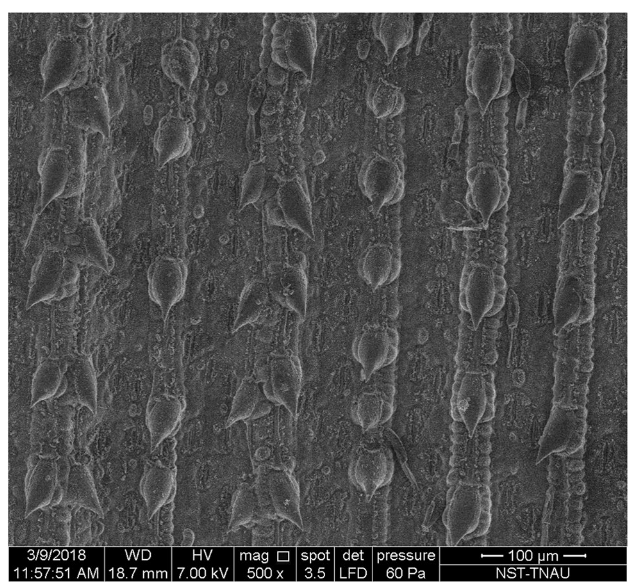

Fig. 2 Observation of sugarcane trichomes density under scanning electron microscope

Table 5 Documentation of mandibular wear on sugarcane early shoot borer as influenced by different sources of silicon

Treatments	Length of the incisor (µm) at		Pooled data
	Poovanthi village of Sivagangai district	Silaiman village of Madurai district	
T_1 - Rice husk ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.504	0.501	0.502
T_2 - Rice husk ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.425	0.425	0.425
T_3 - Bagasse ash @ 500 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.484	0.484	0.484
T_4 - Bagasse ash @ 1000 kg ha^{-1} + SSB @ 2 kg ha^{-1}	0.319	0.334	0.327
T_5 - Calcium silicate @ 500 kg ha^{-1}	0.486	0.496	0.491
T_6 - Calcium silicate @ 1000 kg ha^{-1}	0.208	0.211	0.209
sT_7 - Sodium meta silicate @ 500 kg ha^{-1}	0.525	0.544	0.534
T_8 - Sodium meta silicate @ 1000 kg ha^{-1}	0.522	0.509	0.515
T_9 - Untreated check	0.718	0.708	0.713
SEd	0.0550	0.0549	-
CD (P = 0.05)	0.1166	0.1165	

*Mean of three replications

Figures in parentheses are arc sine transformed values

DAP Days after planting, *SSB* Silica solubilizing bacteria

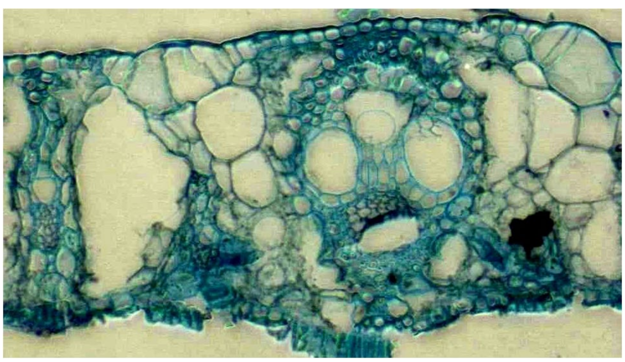

Fig. 3 Observation of cell wall thickness by silicon deposition under phase contrast microscope

0.026 µm due to the basal application of calcium silicate at 1000 kg/ha and baggase ash 1000 kg/ha + SSB at 2 kg/ha respectively whereas in untreated check it was 0.006 µm observed (Fig. 3).

This is in agreement with the findings of [28] reported that silica deposits, commonly called phytoliths, occur in cell walls, cell lumens or in intercellular spaces and external layers and vascular tissues thus providing structural strength to the plant. Kim et al. [29] reported that silicon increases the thickness of epidermic outer cell wall of leaf and leaf sheath [30]. reported that silicon application modifies the cell wall architecture, which was responsible for the increase in the cell wall extensibility. Takahashi [31] reported that silicon deposited in the epidermal tissue may have several functions including support and protection in the form of a mechanical barrier against pathogen and predator invasions.

Interestingly, [6] reported the pattern of silicon deposition in sugarcane especially at the internode and root band enhanced resistance of silicon treated sugarcane plants to *Eldana saccharina*. Similarly, [32] reported that the plants treated with sodium metasilicate @ 2 t/ha recorded cell wall thickness of 589.2µ as compared to untreated check (426.9µ) and acted as a barrier against major pests of rice. Kvedaras and Keeping and Reynolds et al. [33, 34] reported that silicon deposition provides structural rigidity to plants and the resulting physical toughness also makes the plant surface less vulnerable for herbivores to masticate and digest.

3.6 Mandibular Wear

In the present study, the results revealed that the mandibles of early shoot borer larvae were absolutely worn out with minimum teeth length of 0.209 and 0.327 µm which was observed in the treatment with the basal application of calcium silicate at 1000 kg/ha and baggase ash at 1000 kg/ha + SSB at 2 kg/ha as compared to larvae that fed on untreated plants with mandibular teeth length of 0.713 µm (Table 5; Fig. 4a, b).

This is in line with the findings of [35] who reported that the mandibles of larvae of the rice stem borer are damaged due to high silicon content of rice plants. High plant silicon content was a causal factor in larval mandibular wear and that silicon mediated plant defences against the insect herbivores involved were largely mechanical in nature [36]. Sasamoto [25] reported that mandibular wear of lepidopteran larvae is often attributed to plant silicon and has been accounted for the rice stem borer, *Chilo suppressalis* fed on rice. Hanifa et al., 1974 [37] reported that borers feed on silicon fertilized rice or highly siliceous rice varieties

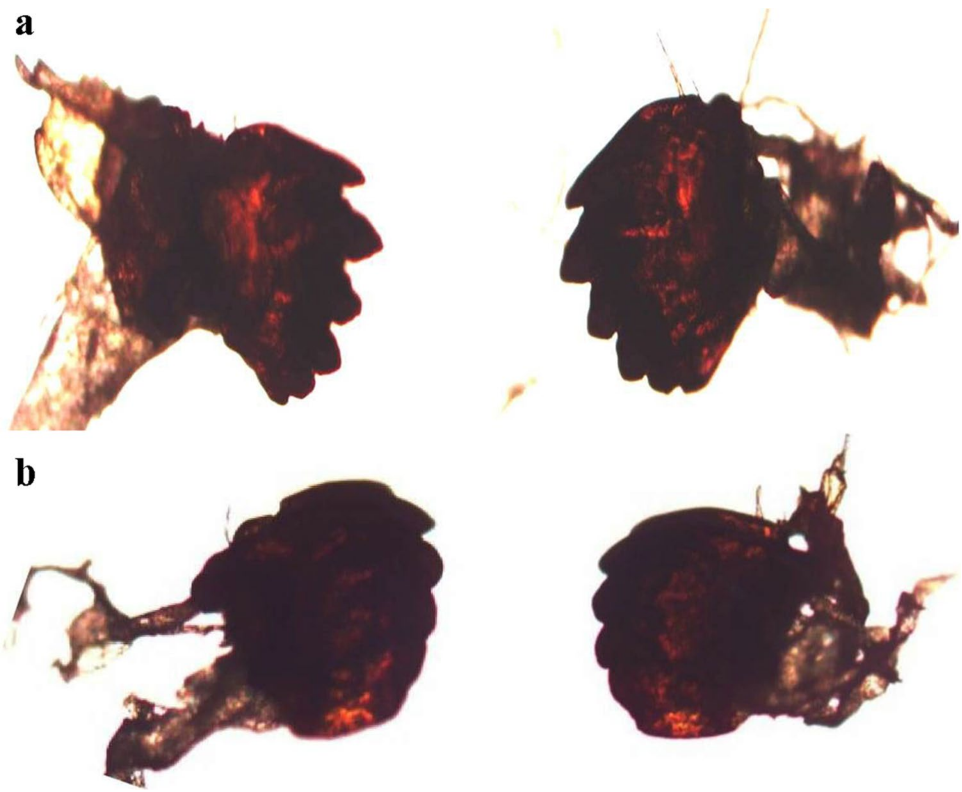

Fig. 4 **a** Mandibles of early shoot borer fed on silicon untreated plants. **b** Mandibles of early shoot borer fed on silicon treated plants

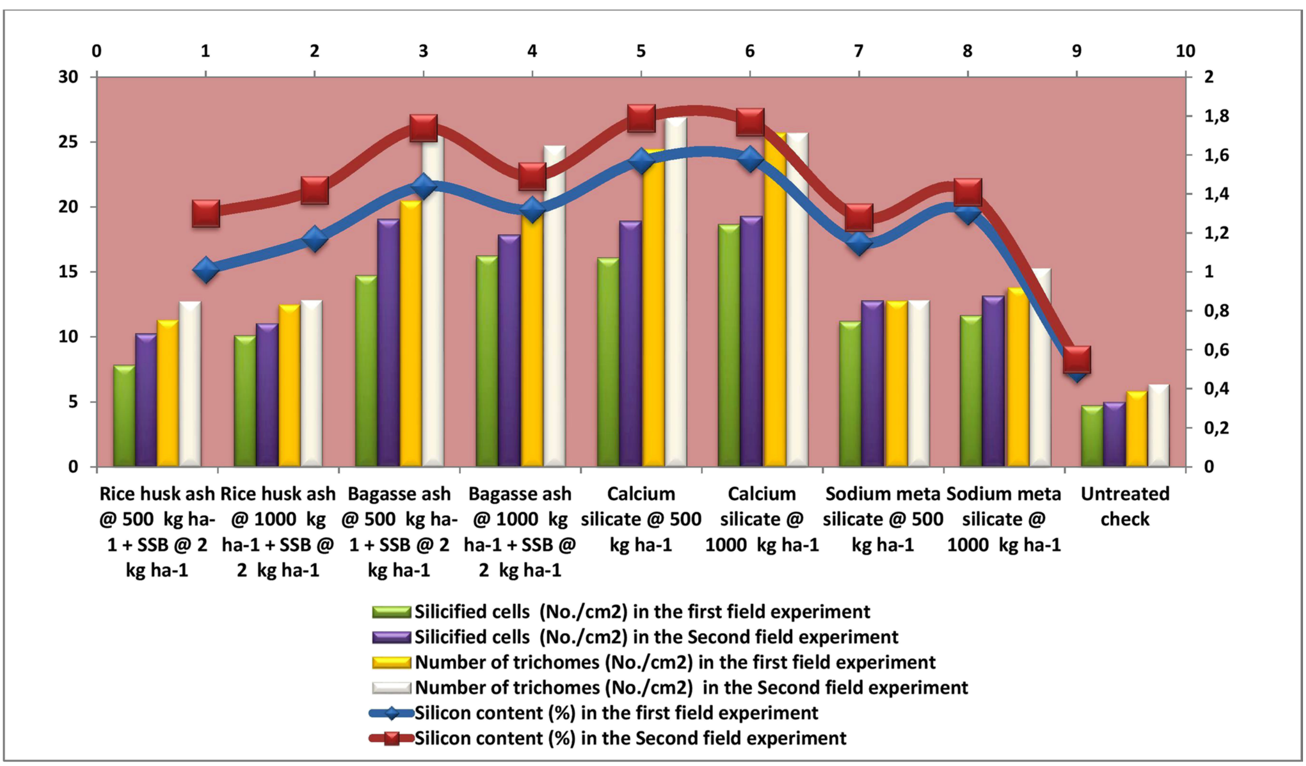

Fig. 5 Correlation between silicon content, silicified cells and trichomes density in the two field experiments

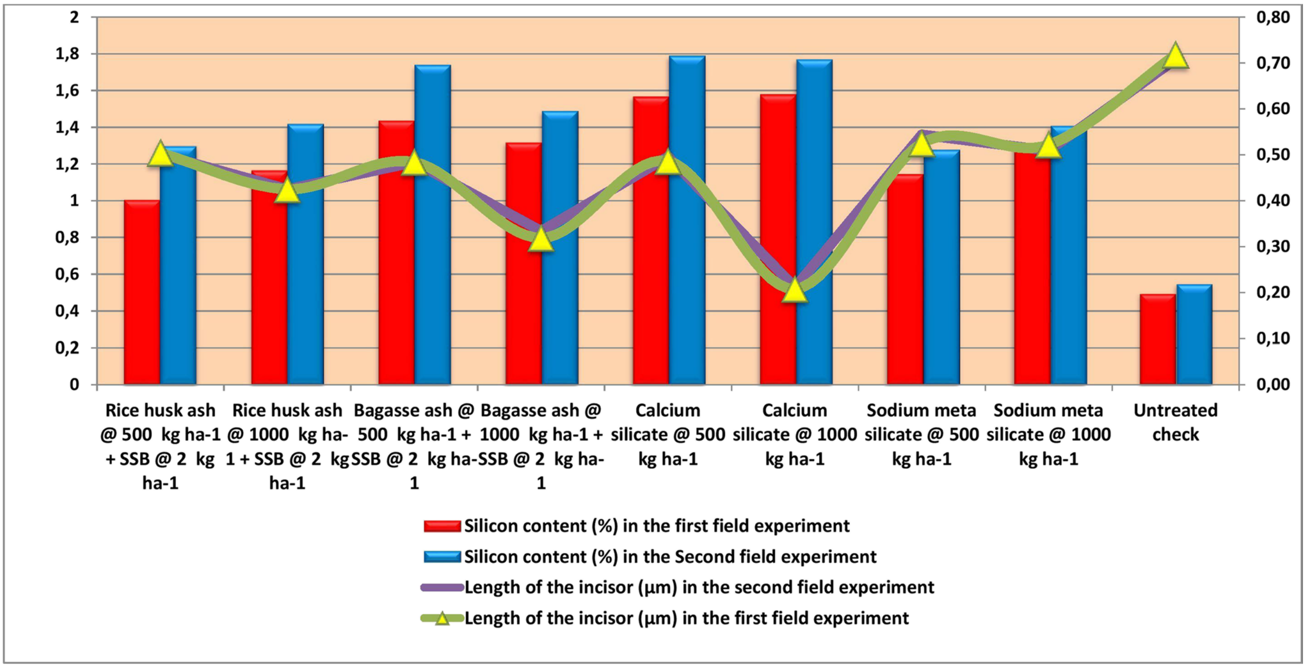

Fig. 6 Correlation between silicon content and length of the incisor of early shoot borer

showed typical effects of antibiosis, such as decreased survival and in some cases worn mandibles indicating reduced feeding efficiency. Goussain et al. [38] reported that wearing out in the incisor region of the mandibles of caterpillar, *Spodoptera frugiperda* Smith when fed on corn leaves fertilized with sodium silicate. The present finding is in confirmation with the findings of [39] who reported larval mandibular wear of *E. saccharina* when fed with calcium silicate treated sugarcane plants (Figs. 5 and 6).

4 Conclusions

Analysis of anatomical factors, through compound and scanning electron microscope (SEM) revealed that application of different silicon sources showed complete wearing of mandibles in sugarcane moderately which can be enhanced by including calcium silicate at 1000 kg/ha as one of the components in IPM, besides for easy adoption by the farmers as it is readily available in the market. Therefore the application of calcium silicate was found to be best option to reduce insect pests of sugarcane and increase cane yield.

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Author Contributions Indhumathi Veeran Sivakumar formulated the research project and with the help of Chandramani Periyakaman, Mahendran Peyandi Parama and Jayachandran Jayaraj conducted field and laboratory experiments and written this article. All authors have read and approved the research article.

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Data Availability All relevant data are within the research paper.

Declarations

Consent to Participate All authors were highly cooperative and involved in research activities and preparation of this article.

Consent for Publication All authors agreed to publish this research article.

Conflict of Interest The authors have declared that no competing interests exist.

Competing Interests The authors declare no competing interests.

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