Silicon (2023) 15:7579–7591
https://doi.org/10.1007/s12633-023-02610-4

RESEARCH

Silicon Mediated Defense Response in Rice Plants Against Brown Plant Hopper *Nilaparvata lugens* (Stål)

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Received: 7 June 2023 / Accepted: 21 July 2023 / Published online: 30 July 2023

Abstract

As silicon is known to have a positive role in enhancing the resistance of rice plants to insects, an investigation on one aspect of the biochemical and molecular basis of rice plant defences mediated by silicon amendments against brown plant hopper *Nilaparvata lugens* (Stål), comprising field, pot culture and laboratory experiments was undertaken in OUAT. Two organic products; Diatomaceous Earth (DAE) at 0.15, 0.30 and 0.45 t/ha, and Rice Hull Ash (RHA) at 2, 3, 4 t/ha, along with one inorganic source, calcium silicate ($CaSiO_3$) at 2, 3, 4 t/ha were soil applied as basal to evaluate their effects on the accumulation of silicon, proline, phenol, carbohydrates and protein in the plant tissues along with proteomic and Scanning Electron Microscope (SEM) studies. Results showed decreasing in proline and protein contents and increasing in silicon, phenol and carbohydrates contents in infected Si amended plants as compared to the control. Proteomic study showed appearing of a thick band of about 20 KDa in infested plants indicating its role in defense mechanism. Under SEM, the dumbbell shaped deposits of Si were marked clearly at different doses of silicon, indicating that the increase in silica dose enhanced its deposits, which was supported by EDAX-SEM data. Results of this study clearly demonstrated that soil amendments with silicon through organic and inorganic sources effectively caused biochemical and molecular changes that ultimately support the plant defenses against BPH.

Keywords *Nilaparvata lugens* · Silicon · Rice plant · Plant defense · Primary and secondary metabolites

1 Introduction

One of the most economically significant insect pests in rice is the Brown Plant Hopper (BPH), *Nilaparvata lugens* (Stl), which can severely reduce production by destroying rice crops [1]. Farmers mostly use chemical insecticides to combat the threat of this pest, but these are costly, labour-intensive, and hazardous to non-target species, leaving the field vulnerable to secondary and resurgence pest infestations [2, 3].

To mitigate these negative effects, there must be a long-term and widely applicable solution, and therefore, integrated pest management (IPM) involving eco-friendly tactics should be developed [4]. One of the most important elements of integrated pest management is host plant resistance. Plant, naturally, exhibits some responses to defence against stresses, including herbivorous insects, which may make it resistant to these stresses [5]. This resistance could be induced in the plant to enhance its defenses and mitigate damage as much as possible. In rice crops, the breeding and cultivation of resistant varieties is economically effective for managing pests, especially BPH [6, 7]. Therefore, to induce host plant resistance, several factors were reported acting as elicitors of biotic or abiotic origin. One of the abiotic tactics that could be benefit to the soil health and rice yield is through amendment of silicate fertilizers [8, 9].

Previous studies have reported the positive role of silicon in enhancing the resistance of rice plants to insects

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by several mechanisms [10–12]. Such those mechanisms are: (1) Physical mechanisms; through deposition of silicon below the cuticle to reduce insect feeding by creating a physical barrier. (2) Biochemical mechanisms via; improve accumulation of defensive compounds in plants e.g. phenolics, flavonoids, proline, phytoalexins etc.; enhancing the activity of defensive enzymes e.g. polyphenol oxidase (PPO), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and phenylalanine ammonia-lyase (PAL); regulation of signalling transduction via phytohormones e.g. salicylic acid (SA), ethylene (ET), and jasmonic acid (JA). (3) Molecular mechanisms; through transcriptomic and proteomic regulation [13–17].

Si is involved in the biochemical and physiological changes in rice plant brought about by the attack of *N. lugens*. Increased callose buildup in the sieve tubes, which inhibits the mass flow of phloem and prevents phloem sap leakage after feeding puncture, is one way that Si induce resistance to *N. lugens* is related [4, 18]. On the other hand, Si amendment can clearly reduce the stress brought on by *N. lugens* by delaying the rise in malondialdehyde (MDA) concentrations, a physiological indicator of stressed plants. By promoting the activity of antioxidant enzymes, Si aids in the scavenging of reactive oxygen species (ROS). The activities of PPO and PAL start and catalyse the oxidation of phenols to quinines right away after an *N. lugens* assault, and this may lessen the acceptability of the insect to plant tissues and ultimately stop the growth of insects [18, 19].

However, between different defensive compounds triggered by silicon amendments in BPH- infested rice plant, little studies have been conducted on primary and secondary metabolites via; proline, phenol, carbohydrate and protein contents in addition to silicon deposits. Keeping this in view, the investigation of this study was to ascertain a side of the biochemical and molecular basis of silicon induced resistance against BPH through evaluating the changes in the mentioned compounds' contents with soil Si amendments.

2 Materials and Methods

In order to test the hypothesis of the present investigation, field experiments along with pot culture and laboratory studies were undertaken at Odisha University of Agriculture and Technology (OUAT) during the year 2016 and 2017 to achieve the objectives relating to impact of exogenous application of "Si" in the rice variety *TN1* for inducing resistance against brown plant hopper under acidic soils of Odisha. The normal recommended agronomic practices have been followed for conducting field and pot culture experiments. The field and pot experiments were laid out in Randomized Complete Block Design with ten treatments and three replications. All the treatments were applied as basal amendments. The detailed list of treatments is depicted below (Table 1).

For biochemical analysis, the plant samples were collected from both the field and pot culture at vegetative and reproductive stage for estimation of the following parameters in the laboratory:

2.1 Silicon Content

The method proposed by Wei-min et al. [20] was used to assess the silicon content of rice leaves. An entire plant was taken from each experimental unit (replicate) and dried in an oven for 7 days at 70 °C. Each sample was crushed and put through a sieve with a mesh size of 60. The samples were dried once again for 48 h at 60 °C. For the purpose of silicon analysis, a sample weighing 100 mg was taken from each treatment and replication. Each 100 mg sample underwent pre-treatment and was stored in a 100 ml polyethylene container. It was then given 3 ml of 50% sodium hydroxide and given a loose-fitting cap. After being gently vortexed, the polyethylene tubes were autoclaved at 121 °C for 20 min. After that, the mixture was poured into a 500 ml volumetric flask, and the volume was adjusted using double-distilled water. One millilitre of the sample solution was transferred to a 50 ml volumetric flask to determine the silicon concentration. Then, 10 ml of ammonium molybdate solution (54 g/L, pH 7.0) and 30 ml of 20% acetic acid were added to it. The mixture was thoroughly shaken and left for 5 min. Five minutes later, five millilitres of 20% tartaric acid and one millilitre of reducing solution were added. The volume was then modified with 20% acetic acid to 50 ml. After 30 min, the solution was preserved, and a spectrophotometer was used to measure the absorbance at 650 nm. Then, as proposed by Wei-min et al. [20], the silicon content of each sample was determined by plugging the O.D. value into the linear regression equation generated from the standard curve of various concentrations of pure SiO_2.

Table 1 Treatments of field and pot culture experiments

Treatment No.	Test products	Source	Dose (t/ha)
T_1	Diatomaceous earth (DAE)	Organic	0.15
T_2	Diatomaceous earth (DAE)	Organic	0.30
T_3	Diatomaceous earth (DAE)	Organic	0.45
T_4	Calcium silicate ($CaSiO_3$)	Inorganic	2.0
T_5	Calcium silicate ($CaSiO_3$)	Inorganic	3.0
T_6	Calcium silicate ($CaSiO_3$)	Inorganic	4.0
T_7	Rice hull ash (RHA)	Organic	2.0
T_8	Rice hull ash (RHA)	Organic	3.0
T_9	Rice hull ash (RHA)	Organic	4.0
T_{10}	Untreated check	-	-

2.2 Proline Content

Following the approach Bates et al. [21], the proline content of rice leaf samples was determined. 0.5 gm of rice leaf samples were homogenised with 3% sulphosalicylic acid for the purpose of measuring proline, and the homogenate was then filtered through Whatman's No. 2 filter paper. After reacting for 1 h at 100 °C with 2 ml of the filtrate, 2 ml of acid-ninhydrin, and 2 ml of glacial acetic acid in a test tube, the reaction was stopped by placing the test tube in an ice bath. 4 ml of toluene was used to extract the reaction mixture, and the toluene-containing chromophore was pipetted out and allowed to cool to room temperature. At 520 nm, the produced color's absorbance was measured against a blank for the reagent. Following the aforementioned technique, a series of Standard proline solutions (20–100 µl) concentrations were made against the material.Proline concentration is measured in mg/g (F.W. (Fresh Weight)) of tissue.

2.3 Total Phenols

The method of Bray and Trope [22] was used to determine the total phenols in rice leaf samples. In a mortar and pestle, a 0.5 g sample of leaves was ground with 10 times the volume of 80% ethanol. Centrifuging the homogenate at 10,000 rpm for 20 min. The residue was again extracted with five times the volume of 80% ethanol after the supernatant had been collected in a 30 ml test tube. The residue was dissolved in 5 ml of distilled water, and 1 ml of the ethanolic extract was mixed with 0.5 ml of the Folin-Ciocaltaeue Reagent (FCR). After three minutes, two ml of a 20% sodium carbonate solution was added, and the mixture was thoroughly stirred. After precisely one minute in a boiling water bath, the tubes were removed and allowed to cool to room temperature. At 650 nm, the produced color's absorbance was measured against a blank for the reagent. As stated above, a series of Standard catechol solutions with concentrations ranging from 10 to 50 µl were created against the sample. The amount of phenols present in plant tissue is measured in mg/g (F.W.).

2.4 Total Carbohydrate

Following the Anthrone method, the total carbohydrates in treated rice leaf samples were determined [23]. The rice leaf sample, which weighted out to about 100 mg, was placed in a boiling tube and hydrolyzed by being placed in a bath of boiling water for three hours with 5.0 ml of 2.5 NHCl before being allowed to cool to room temperature. After being hydrolyzed, the sample was neutralised with solid sodium carbonate until no longer bubbling. After that, the volume was double-distilled to make it 10 ml, and it was centrifuged at 5000 rpm. 0.1 ml of the supernatant was taken and utilised for analysis. The working standards of glucose were divided into 0.2–1.0 ml portions for the standard curve, and 1.0 ml of water containing anthrone reagent was used as the blank. With double distilled water, the volume in each test tube was brought up to 1 ml. Anthrone reagent was then added, cooked for 8 min in a boiling water bath, and quickly cooled. At 630 nm, the colour green to dark green's absorbance was measured on a white background. The amount of carbohydrates in plant tissue is measured in mg/100 g (F.W.).

2.5 Protein Content

The protein content of rice leaves was determined using the Lowry et al. technique [24]. Small portions of a one-gram sample of rice leaf were cut and weighed. The leaf sample was homogenised in a pre-chilled mortar and pestle at a 1:3 ratio (1 g sample: 3 ml extraction buffer) with 50 mM potassium phosphate buffer at pH 7.8. The homogenised plant sample was placed in an Eppendorf tube, where it was centrifuged twice for 15 min at 15,000 rpm. After that, the supernatant was gathered and kept at 4 °C. 40 µl of the supernatant were obtained and diluted 50 times with distilled water in order to measure the protein. Then, 500 µl of distilled water was added to the 500 µl that had been collected from the diluted supernatant. The above content was added to, and correctly mixed using a vortex/cycle mixer using 4 ml of Reagent C (a mixture of Reagent A (0.1 N NaOH in 2% Na_2CO_3), Reagent B1 (1% $CuSO_4$), and Reagent B2 (2% sodium potassium tartrate) at 96 ml + 2 ml + 2 ml, respectively). This mixture was then let to stand for 10 min. Following this, 0.5 ml of Folin-Ciocaltaeue Reagent (FCR) was added, and the mixture was left in the dark for 30 min to acquire a light blue hue. After 30 min, the produced color's absorbance was measured at 750 nm using a reagent as a blank. Bovine Serum Albumin (BSA) solutions (0.2-1 ml) corresponding to 2.0–10 g concentrations were also examined as before for the standard curve. Protein content is measured in milligrammes per gramme (F.W.) of plant tissue.

2.6 Analysis of Protein Profile Using SDS-PAGE

Distilled water, 40% Acrylamide, 1% bisacrylamide, 375 mM Tris (pH 8.8), 0.18% SDS, 0.05% ammonium persulfate, and 0.4 µl /ml TEMED were combined to create the resolving gel, which was then cast onto the glass plates. After the resolving gel had solidified, stacking gel was made by combining distilled water with 125 mM Tris (pH 6.8), 40% Acrylamide, and 1% bisacrylamide. On top of the resolving gel, 0.1% SDS, 0.05% ammonium persulfate, and 0.05 µl/ml TEMED were cast. The comb was positioned so that wells for loading protein sample were created. Protein samples were combined with a loading buffer that was made by

combining Tris-HCL buffer (pH 6.8), 5% -mercaptoethanol, 20% glycerol, 2% SDS, 667.5 l distilled water, and 0.1% bromophenol blue. The mixture was then heated for five minutes at 100 °C. The sample mixture was then added to the wells along with common protein markers. For 3–4 h, the Gel was operated at 80 volts. The gel was stained with staining solution (Coomaise brilliant blue 0.25%, 40% methanol, 10% acetic acid, and 50 ml distilled water) and left overnight after running for a predetermined amount of time to allow protein migration up to the bottom of the gel with tacking dye (bromophenol blue). After that, a destaining solution (40% methanol, 10% acetic acid, and 50 millilitres distilled water) was applied to destain the gel.

2.7 Scanning Electron Microscope Studies

The samples were collected, after 40 DAT, from all treatments and replicates of infected plants in pot culture experiment and stored in freeze-dried condition at 2 °C. They were rapidly fixed in 3% glutaraldehyde in 0.1 M phosphate buffer solution after being cut into 1–2 cm^2 pieces. They were then coated with grainsize gold particles (1.5-3.0 nm) and inserted in the system running at 20 kV after being dehydrated through a graded sequence of ethanol [25]. Field Emission Gun (FEG)-SEM (JEOL JSM-7600 F) was used to create the coloured SEM-EDX micrographs, while the Hitachi S-3400 N VP-SEM was used to create the grayscale images.

2.8 Statistical Analysis

The data generated from various field and pot culture experiments were subjected to statistical analysis for proper interpretation. Data were subjected to suitable analysis as suggested by Gomez and Gomez [26]. All the biochemical data viz., carbohydrate, phenol, proline, protein, silica analysis were calculated based on the respective standard curve and the contents for various plant samples were determined and expressed as mg/gm or mg/100 gm F.W. of plant tissue. Data was analysed using SPSS program, and DMRT analysis was used for Post- Hoc test.

3 Results and Discussion

Organic or inorganic silicon can be supplied to the plant, absorbed as uncharged silicic acid Si(OH)4, and eventually irreversibly precipitated throughout the plant as amorphous silica, where some biochemical and molecular changes are occurred which, as a result, serve the plant defenses [27]. The responses to silicon supplements are most evident in plants that accumulate silicon, such as rice. We discuss these changes below:

3.1 Silicon Content

Efficacy of silicon amendments on Si accumulation in rice plant grown under field and pot conditions was exhibited in Fig. 1. Silicon amendment at different doses through organic and inorganic sources had significantly increased the silicon content in rice plants grown in field and pots. In Field experiment, as well as in pot one, treatments followed the same trend; in both of DAE and RHA, the Si content increased as the dose increased. Same thing was there in $CaSiO_3$ with the highest deposit recorded in its medium dose T_5 (10.28, 9.13 g/kg) as compared to the control (5.02, 4.49 g/kg) in field and pot experiment, respectively. Here, all the three sources of silicon amended at low, medium and high doses resulted in significant increase in Si content in rice plants over control as evident from both field and pot culture trials. Plants with silica additions have leaf sheaths with a 10 times greater Si content than plants without treatments [15]. This significant accumulation of Si by rice plants points to its critical function in modulating *Oryza* spp.'s resilience to diverse biotic stresses [28, 29]. A silico- cuticular double layer of a 2.5 µm thick is formed beneath the cuticle of the rice leaf, and this layer, which is the most prevalent type of silicon deposit in leaf blade and sheath, is largely impervious to insect proboscis penetration [30].

3.2 Proline Content

The proline content, either in organic or inorganic Si sources, significantly increased in infected leaves at the vegetative and reproductive stage more than that in the healthy leaves, as shown in Fig. 2. At vegetative stage, the highest Si accumulation was observed in RHA treatments; T_7, T_8 and T_9, which significantly had the same effect of (10.58, 10.37 and 10.40 µmoles/g), respectively. While at reproductive stage, the highest proline content was recorded in T_3 (11.88 µmoles/g). Most of the treatments exhibited increasing in proline content with the increase of the dose. The proline content significantly increased in control compared to other infested treated samples due to more secretion of amino acid in plants treated without silicon. Proline has various roles in plants under varied stress conditions, operating as a metal chelator, an antioxidant defence molecule, and a signalling molecule [31]. Many plant species accumulate proline while under stress as an adaptive reaction to unfavourable circumstances [32, 33]. As a response to insect attack, a signalling pathway related to increase in proline synthesis is triggered in infected plants as defensive reaction [34].

Si supplementation, under salinity stress conditions, resulted into reducing of proline content in wheat plants to a level where the stability of the cellular plasma membrane is kept by maintaining the redox equilibrium along with other biochemical compounds [35]. This indicates that

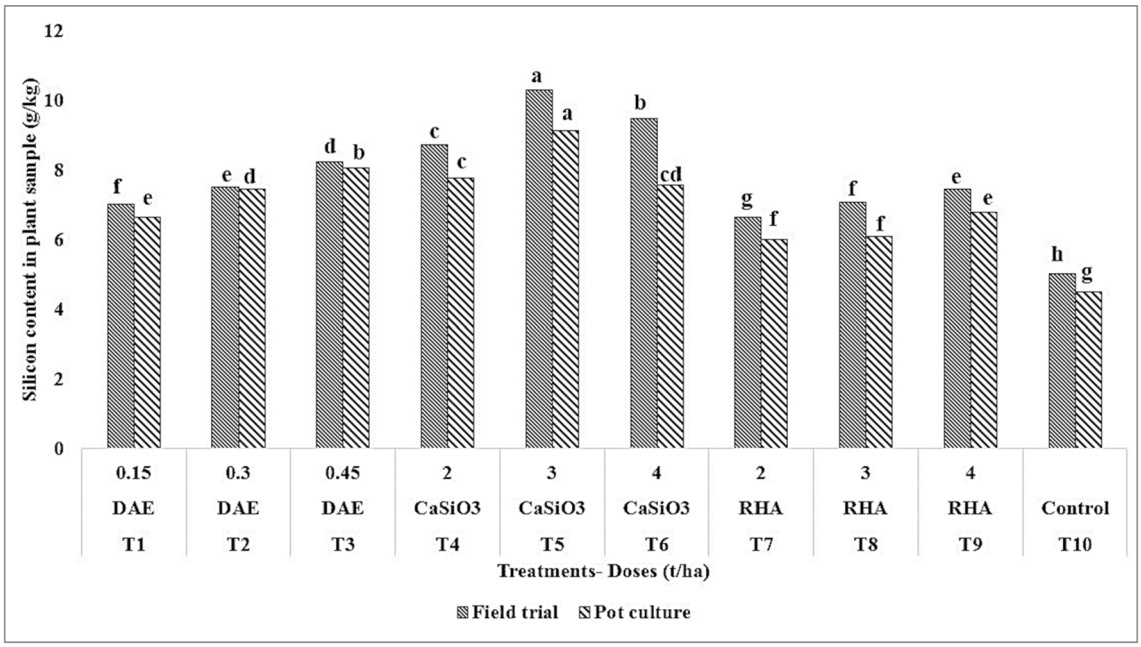


Fig. 1 Efficacy of silicon amendments on Si accumulation in rice plant grown under field and pots in cv. *TN1*- Graphs are made using Excel- DMRT's post hoc test; Bars with same alphabets are significantly indifferent ($p < 0.05$)

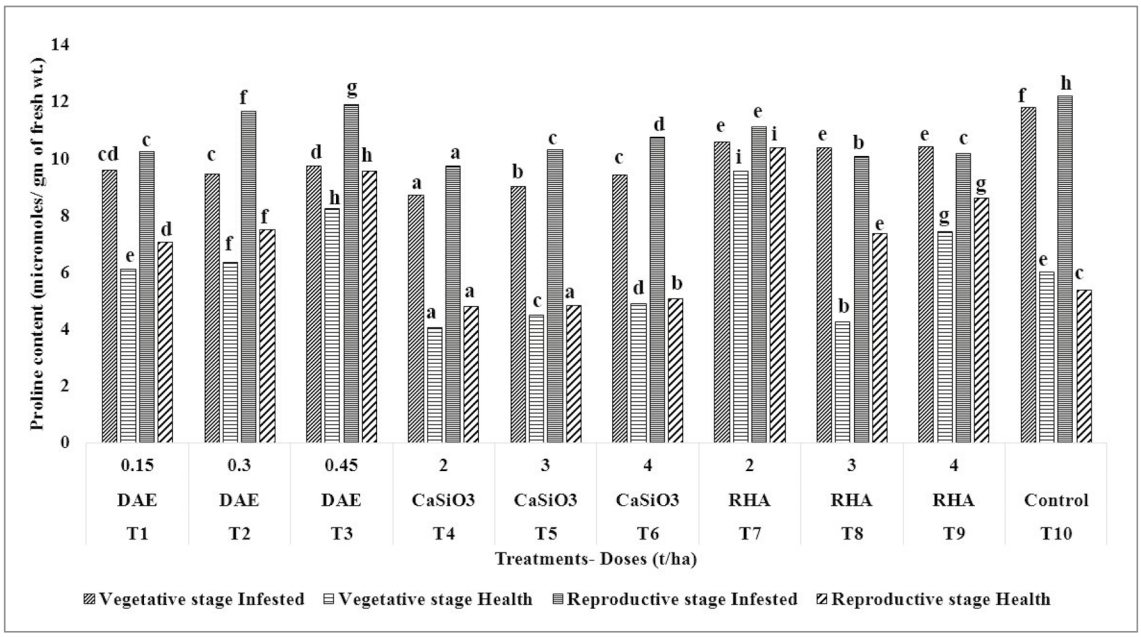


Fig. 2 Effect of various doses of silicon on proline content in rice var*TN1* grown in pots, with and without BPH, at vegetative and reproductive stages- Graphs are made using Excel- DMRT's post hoc test; Bars with same alphabets are significantly indifferent ($p < 0.05$)

silicon regulates the increasing level of proline content under stress. And this decrease in proline content compared to the control is only to achieve balance and integration with other defensive compounds to serve the ultimate goal of stimulating plant defenses against stress and reducing stress damage compared to plants that didn't receive Si amendments. It was pointed that Si can act as a modulator affecting the timing and extent of plant defense responses [10]. Under healthy condition, except the inorganic source, the other two products resulted in greater accumulation of proline during

vegetative and reproductive stages. The reasons for lower proline accumulation in plants receiving inorganic source of Si needs to be ascertained through further studies.

3.3 Phenol Content

The production of phenols in plants was influenced by the application of silicon through different sources as presented in Fig. 3. The phenol content was more in the infested plants than that in the healthy plants, irrespective of the treatments. Phenol content varied from (7.23–12.21 mg/gm) in infested plants at the vegetative stage as compared to control (6.38 mg/gm). At the reproductive stage, the phenol content was ranged from (9.10 to 12.68 mg/gm) in infested plants as compared to control (8.77 mg/gm). However, in all of the infested samples, at both stages, except T_3 and T_4, there was no differences in phenol content among treatments, as compared to T_{10} which was as par as of T_3 and T_4. Phenols are plant secondary metabolites which have several health and defensive properties [36]. In addition to protect plant against some stresses like oxidative stress, phenolic compounds are also involved in plant defensive response to insect attack [37, 38]. Phenols physically minimize insect infestation by making leaves more robust, and they also serve as inhibitors and toxins that discourage herbivores from feeding on them [39, 40]. Here, Si amendment treatments have resulted into significant increase in phenol contents over control. It was proved that Si amendments enhance the amount of phenol in plant under biotic stress which shows defensive properties [28, 41]. High dose of DAE resulted in less accumulation of phenols. Several studies have suggested that silicon- accumulating plants have lower amounts of phenol, because these plants replace carbon with silicon-based defenses, partially. This may explain the decrease in phenol content in rice, which is a silicon accumulating- plant, at a high dose of silicon additives [42–44]. One study suggested the presence of a negative correlation between silicon and phenol, and that a lower dose of silicon may result into a better effect of phenol against insects [45].

3.4 Carbohydrate Content

Carbohydrate content at vegetative stage revealed that there is a distinct variation between the treatments with a record of a maximum content in T_3 (14.51 mg/100 g) and a minimum one in T_6 (11.22 mg/100 g) in infested plants as against (9.59 mg/100 g) in control (Fig. 4). Similarly at reproductive stage in infested plants, where the maximum content was recorded in T_3 and T_7 (13.69 mg/100 g) and (13.22 mg/100 g), respectively, and minimum one (8.17 mg/100 g) in T_6 as against T_{10} (7.43 mg/100 g). Silicon amendment has brought about a marked increase in the carbohydrate content in rice plants with a strong evidence of greater carbohydrate accumulation in response to BPH feeding in comparison to normal healthy plants. The increase in carbohydrates content in infected rice plants points to their potential significance in signalling pathways that activate plant defence mechanisms. To improve defence against insect pests, plants modify their photosynthesis and subsequently carbon fixation. A substantial rise in total

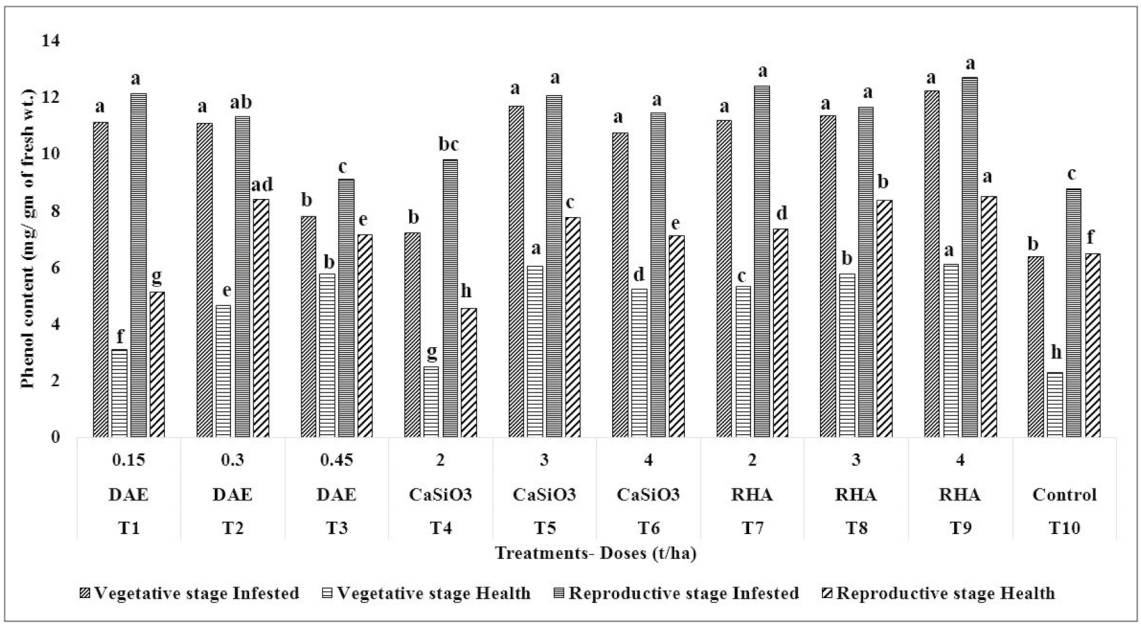


Fig. 3 Effect of various doses of silicon on phenol content in rice var*TN1* grown in pots, with and without BPH, at vegetative and reproductive stages- Graphs are made using Excel- DMRT's post hoc test; Bars with same alphabets are significantly indifferent ($p < 0.05$)

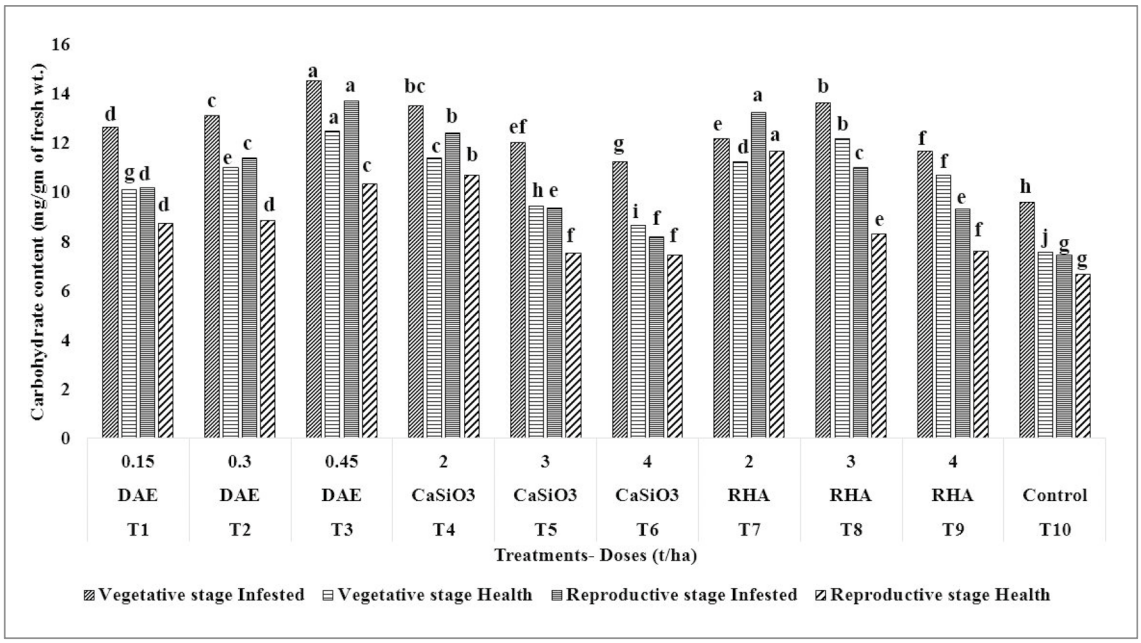


Fig. 4 Effect of various doses of silicon on carbohydrate content in rice cv. *TN1* grown in pots, with and without BPH, at vegetative and reproductive stages- Graphs are made using Excel- DMRT's post hoc test; Bars with same alphabets are significantly indifferent ($p < 0.05$)

non-structural carbohydrates in Lucerne is reported in plants infected by potato leafhopper, *Empoasca fabae* (Harris) as compared to healthy plants [46]. It was observed in several studies that the application of silicate fertilizers in an under stressed- field enhances the content of carbohydrate in a manner dependent on dose [47–49]. The harmful activity of ROS increases under stress, resulting into increasing in carbohydrate oxidation which reduce its concentration, and as silicon can scavenge the oxidative activity of ROS, then the carbohydrate content will increase compared to un-Si amended plants [50–52].

3.5 Protein Content

As shown in Fig. 5, in most of the treatments, protein content was lower in infested plants than healthy ones, and same thing was there in control. Also, Si amendments in all of the treatments resulted into decreasing in the protein content as compared to the control. At vegetative stage, in infested plants, T_8 (24.24 mg/gm) had the highest protein accumulation and T_4 had the lowest one (11.97 mg/gm) as compared to the control (26.38 mg/gm). Infested plants, at reproductive stage, exhibited similar trend with maximum protein content in T_8 (15.55 mg/gm) and T_2 (15.53 mg/gm), where T_4 resulted into the minimum protein accumulation of (10.70 mg/gm) as against (16.85 mg/gm) in T_{10}. Under stresses, such as an insect attack, alterations in gene expression take place leading to changes in protein quality and quantity, which have a crucial role in signal transduction and oxidative defence [53, 54]. According to Mishra [48] Exogenous silicon treatment to rice plants, infected by rice leaf folder, resulted into a noticeably higher level in activity of Trypsin protease inhibitor as compared to non- Si-treated plants. Han et al. [13] reported that the concentration of soluble protein in leaves is negatively impacted by Si supplementation. The amount of Si and carbohydrates accumulated in Si treated plants are inversely linked with this decreased protein accumulation. This might be because Si addition encourages photosynthesis, increasing sugar concentration while decreasing nitrogen concentration, leading to a high ratio of C: N [55–57]. Also, it is known that silicon interferes with the signalling transaction and phosphorylation processes by binding to the hydroxyl groups of amino acids associated with those processes, and thus may affect the activity and conformation of protein [10].

3.6 Analysis of Protein Profile Using SDS-PAGE

To investigate the role of defense responsive protein in healthy and BPH infested rice plants receiving Si amendments at various doses, SDS-PAGE analysis of rice leaf was carried out. Figure 6 showed the protein profile assessment of healthy and infected plants with BPH, at vegetative and reproductive stages, under the application of different doses of DAE, Calcium silicate and RHA.

In the vegetative phase, T_1 in healthy plants showed 12 protein fragments ranged from 1 KDa to 96KDa. Under the same treatment, in the infected plant 11 protein fragments

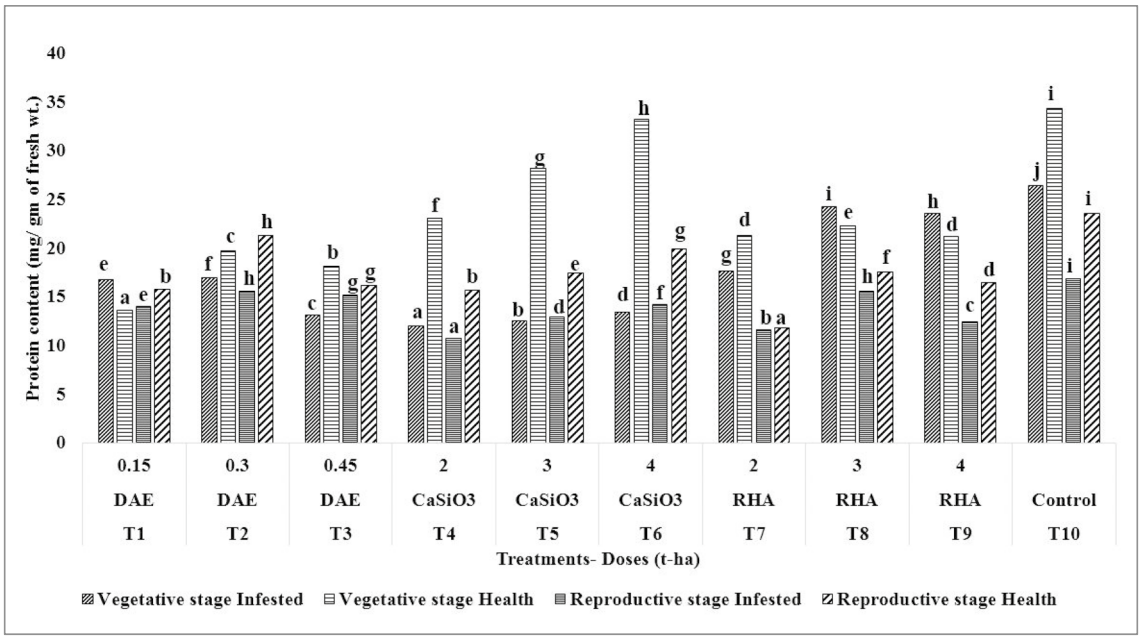


Fig. 5 Effect of various doses of silicon on protein content in rice cv. *TN1* grown in pots, with and without BPH, at vegetative and reproductive stages- Graphs are made using Excel- DMRT's post hoc test; Bars with same alphabets are significantly indifferent ($p < 0.05$)

appeared and one protein fragment in a size of 43 KDa disappeared. However, similar results was also observed in both healthy as well as infected plants with T_2 and T_3. In the case of calcium silicate application (T_4, T_5 and T_6), there was no variation with regard to protein profile in healthy and infected plants. There were 10 protein fragments appeared in both healthy and infected plants, ranged from 0.5 KDa to 96 KDa but the thickness of the fragment was denser in T_6. In case of RHA application (T_7, T_8 and T_9), there was 12 protein fragments that appeared in both healthy and infected plants. There was no significant difference on the basis of the qualitative analysis of the protein profile. In control healthy and infected plants produced 14 fragments. There were no significant differences between healthy and infected plants.

During reproductive stage, with application of DAE (T_1, T_2 and T_3), both healthy and infected plants showed 14 fragments ranged from 1 KDa to 96 KDa. There was no significant variation among the healthy and infected plants under DAE treatments. With the application of either calcium silicate or RHA, there was no significant variation and 13 fragments in both healthy and infected plants were appeared. In all the cases, the infected plants showed a thick band of about 20 KDa as compared with healthy plants. This indicates that during infection, the plant produces more amount of stored protein with a size of 20 KDa to help in the defense mechanism.

Relative quantification proteomic analysis using high sensitivity mass spectrometry technology is becoming increasingly popular due to its great sensitivity and reproducibility properties [58]. A total of 10–14 protein bands of 0.5–96 KDa were observed in different treatments including control. With appearance of 11 protein fragments, DAE showed its prominence by absence of the 43 KDa protein band in infested samples particularly at higher doses. The specific role of this protein needs to be ascertained for better understanding of greater level of resistance due to DAE application. Plants receiving calcium silicate and RHA at different doses showed 10 and 12 fragments, respectively without any variation between healthy and infested ones. Hence, there was no qualitative difference in protein content, however, the darker fragments indicates greater accumulation of specific protein at higher doses. At reproductive stage no marked variations observed between doses so far as protein fragments are concerned. But prominence of 20 KDa fragment in infested plant sample suggest its probable involvement in plant defense mechanism through greater accumulation due to insect feeding.

3.7 Scanning Electron Microscope Studies

Silicon deposition on rice leaf of infested plants were observed under Scanning Electron Microscope (SEM) at 2000x magnification which enabled us to compare the deposits per 20 µm surface area (Fig. 7). At different doses of silicon, the dumbbell shaped deposits seemed to become more intensified and prominent. A distinct difference in silicon deposition can be marked between control and other doses, giving us an idea that an increase in silica dose,

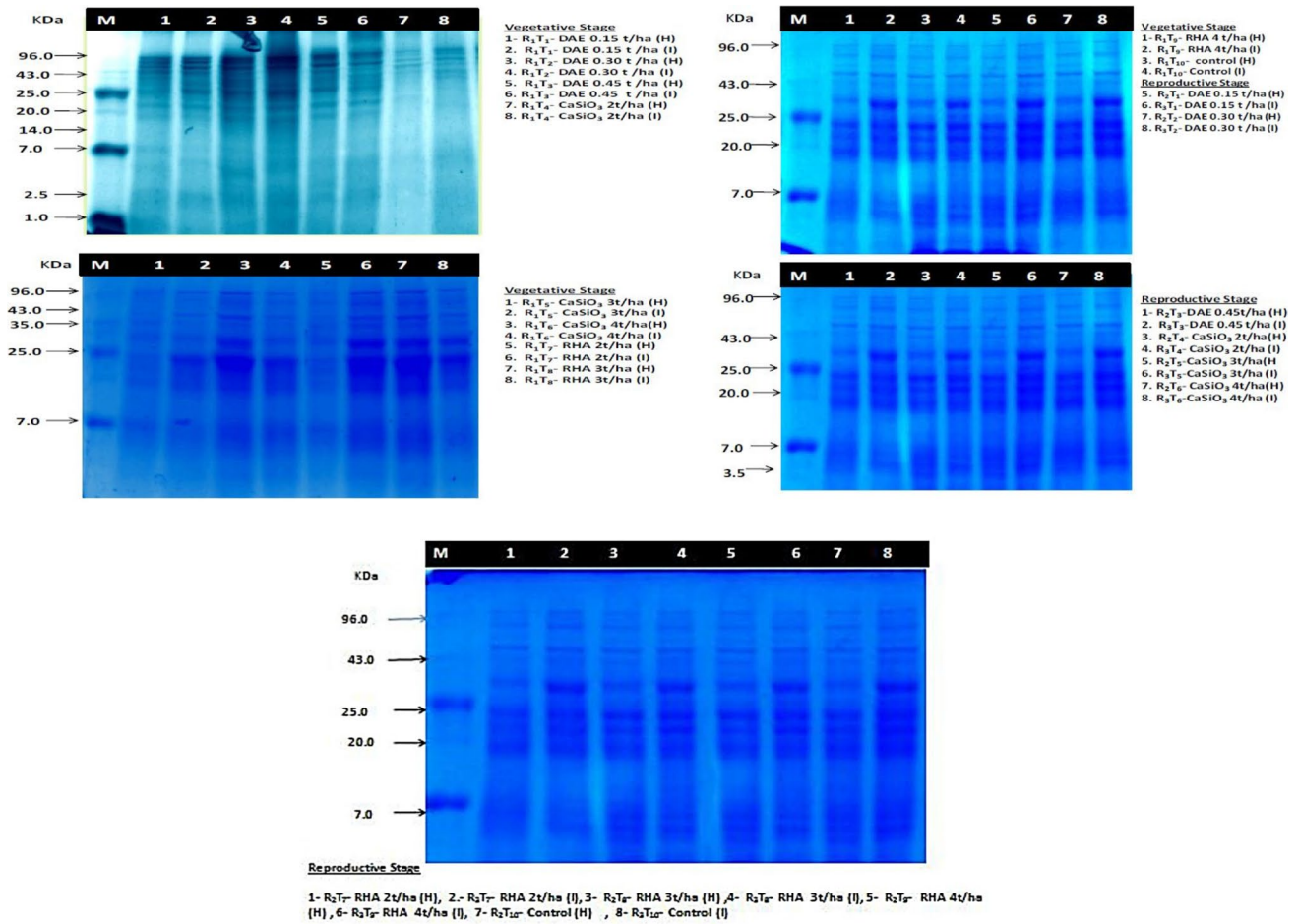
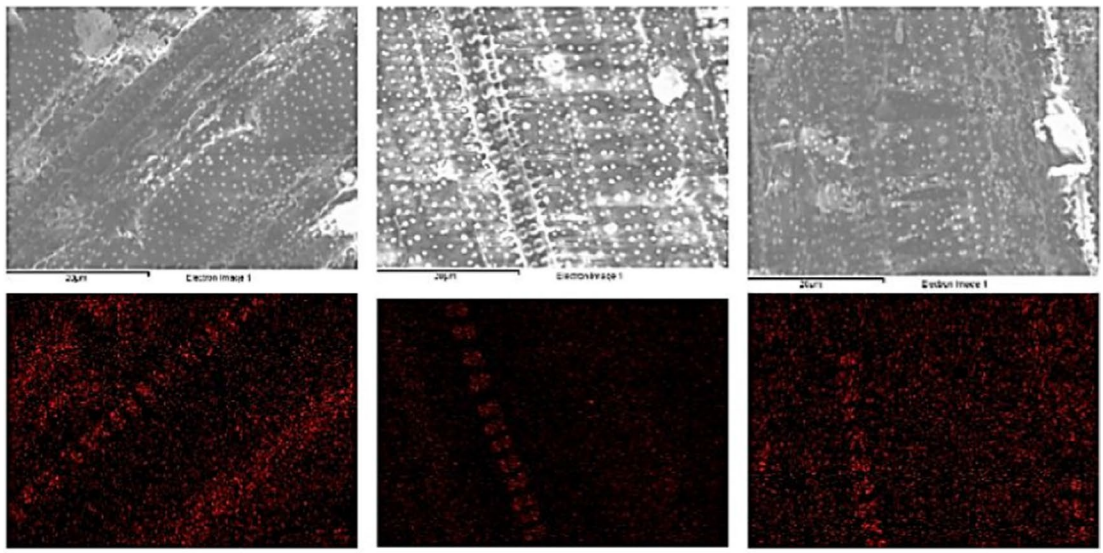


Fig. 6 Protein profile of healthy (H) and infected (I) plants grown in the pot, at vegetative and reproductive stages, with different doses of DAE, RHA and $CaSiO_3$

enhanced its deposits. The observation was strengthened by the Si weight% data obtained from EDAX-SEM (Energy Dispersive Analysis of X-rays- Scanning Electron Microscope) wherein the Si atomic weight% in DAE @ 0.15 t/ha, DAE @ 0.45 t/ha, CaSiO3 @ 2 t/ha, $CaSiO_3$ @ 4 t/ha, RHA @ 2 t/ha, RHA@ 4 t/ha and that of control, were 9.82, 10.27, 9.05, 9.25, 8.46, 8.73 and 4.41 per cent, respectively. The graph obtained also depicted the high content of silicon in higher doses with respect to control.

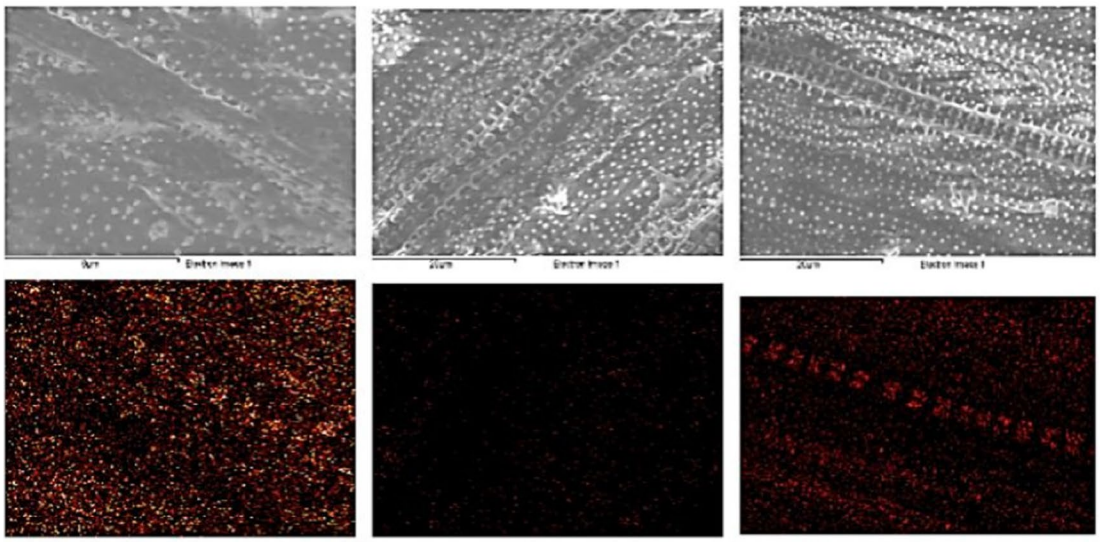
Scanning electron micrograph investigation revealed that Si addition led to intensive cell silicification in rice leaves with the enhanced deposit of small silica cells along with ladder like dumbbell shaped silica bodies distributed in rows along the vein (Fig. 7). Grey images represent Si deposit on leaf surface under scanning electron microscope at 2000x magnification whereas the dark images with fluorescent red granule depicting Si concentration detected through Electron Density X- ray spectroscopy (EDX). The suggestion that enhanced silica deposition is linked to the ability of the rice plant to combat biotic stress is supported by this SEM and EDX analysis. The number of silica cells and silica body per unit length were markedly higher in Si amended plants and varied in a dose dependent manner as against a sparsely distributed silica bodies in control sample. Thus, Si content in rice plants and silica cells seems to have a strong positive correlation. However, the density of Si cells does not show a true relation with Si content indicating probably the presence of bulliform cells forms the real barrier for insect probing rather than concentration of Si small cell on leaf surface. According to Dorairaj and Ismail's reports, as silicon content rises, the deposition of silicon may switch from tiny cells to bulliform cells. And these microstructures, as it is hypothesised, give the rice plant strength and serve as a barrier to defence against plant hoppers [29, 59]. It is well known how Si build-up in epidemic cells creates a physical barrier that protects plants from herbivores, providing plant resistance to them [13, 56, 60]. In Si-treated rice plant, the formed physical barrier after insect attack is multiplied approximately 10 times, due to the changes of the Si concentration and the histological parameters of the silica cell in the leaf sheath



T_1: DAE (Low dose)
U-Si deposit, L-Si density

T_4: $CaSiO_3$ (Low dose)
U-Si deposit, L-Si density

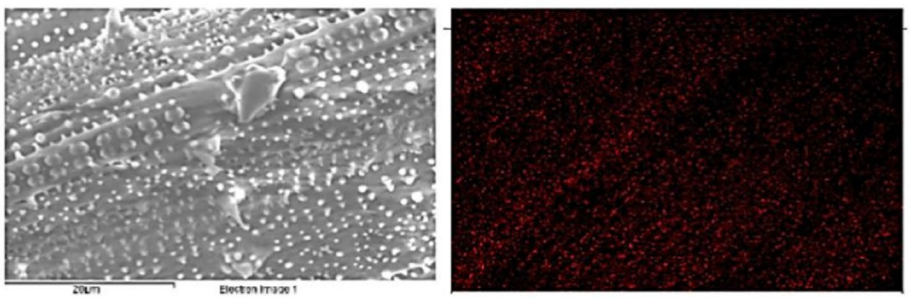
T_7: RHA (Low dose)
U-Si deposit, L-Si density



T_3: DAE (Low dose)
U-Si deposit, L-Si density

T_6: $CaSiO_3$ (Low dose)
U-Si deposit, L-Si density

T_9: RHA (Low dose)
U-Si deposit, L-Si density



T_{10}: Control Left: Silica cell deposit Right: Silicon cell density

Fig. 7 Deposition of silicon in leaves of rice plants receiving different doses of silicon amendments with EDX mapping

[15, 61]. This strengthening provided by Si addition to the plant creates difficulties for the insect to penetrate the plant and makes the pest more vulnerable to natural enemies, as was reported in sugarcane stalk borer case and this may represent a complementary approach between integrated pest management and silicone applications [61, 62].

4 Conclusion

To ensure sustainable rice production, it is always essential to develop ecologically sound alternative methods. Si amendment may be one such potential alternative. Results of this study clearly demonstrated that soil amendment with silicon through organic and inorganic sources effectively caused biochemical and molecular changes that ultimately support the plant defenses against BPH. Hence, this study has furthered the understanding on mechanism of Si mediated resistance to brown plant hopper in rice. All the three sources via; DAE, CaSiO3 and RHA at low, medium, and high doses exhibited different level of defensive biochemical/ molecular regulations against this hopper pest, which can be commercially exploited for wider use in agriculture.

Acknowledgements This work was supported by grants from Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India.

Author Contributions Subhalaxmi Roy carried out the experiment, recorded data and wrote the manuscript. Reem Mohammad has helped in drafting the manuscript. Deepak Kumar Swain has analysed some part of data. Bhagyashree Khamari and SP Monalisa have visualised writing review and editing. All authors have read and approved the final manuscript.

Data Availability Not applicable.

Declarations

Ethics Approval Not applicable.

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.

Competing Interests The authors declare no competing interests.

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