Determination of Silicon Accumulation in Non-Bt Cotton (Gossypium hirsutum) Plants and Its Impact on Fecundity and Biology of Whitefly (Bemisia tabaci) under Controlled Conditions

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Abstract: Considering the resistance development-potential of whitefly (Bemisia tabaci) against control tactics with limited action mechanisms, the present study investigated the accumulation of two different silicon (Si) sources (SiO2 and K2SiO3) in cotton plants. The tested dose rates (0, 200, and 400 mg/L) of both Si sources were applied directly to the soil or through foliar application on cotton leaves. Moreover, a laboratory bioassay was also conducted to evaluate the performance of applied Si sources against the oviposition preference and biology of B. tabaci. A significantly higher Si accumulation, reduction in oviposition preference, and prolonged developmental period of all nymphs and total life cycle of B. tabaci was observed in the case of foliar-applied silicon. Similarly, among Si sources, a significant decline in the number of oviposited eggs and delay in the developmental period of B. tabaci was observed in the case of SiO2 followed by K2SiO3. Moreover, cotton plants subjected to SiO2 treatments possessed higher Si contents in their leaves than K2SiO3 treated plants. The results further revealed that both Si sources showed promising results at their higher concentrations regarding the tested parameters of Si accumulation, fecundity, and developmental period of B. tabaci. Our results strongly suggest that among emerging pest control strategies in cotton plants lies the use of foliar application of Si, which can also be incorporated in different integrated pest management programs due to its safety for humans and beneficial insect fauna.

Keywords: Bemisia tabaci; developmental duration; silicon accumulation; oviposition preference

1. Introduction

Plants attacked by insect herbivores have the potential to reconfigure their metabolism [1,2] by entailing certain induced defense responses such as the reallocation of primary metabolites [3–5] as well as the production of secondary metabolic compounds [6,7].
In addition, to directly affect the attacking herbivores, these defense responses also determine the later fate of the plants in subsequent colonization by additional herbivores or by avoiding further pest invasion. These plant responses induce certain changes in the nutritional quality and defense chemistry of the host plant as a resource and also regulate the production of certain volatile compounds that foraging herbivores perceive to locate its potential hosts [8–11]. In most cases, these metabolic changes increase plant resistance to insect feeding and discourage successive pest attacks [6,9,12]. However, in some cases, prior feeding diminishes plant resistance or encourages subsequent herbivory [13] as some insects, including whiteflies [14], utilize damage-associated cues for aggregative feeding [10,15,16].

The whitefly *Bemisia tabaci* (Gennadius; Homoptera: Aleyrodidae) is a notorious cosmopolitan pest of several economically important field and greenhouse crops and has become a major threat to global food security [17,18]. It directly causes severe damage to the crops by sucking cell fluid, inducing various physiological disorders, and indirectly by vectoring more than 200 viruses, including the notorious cotton leaf curl virus [19–21]. Whiteflies feed cryptically in large aggregations from the underside of the leaves and overcome plant defense responses [16,22]. Aggregative feeding behavior may lead to nutrient competition resulting in the operation of more severe plant responses [23], including initiation of the jasmonic acid pathway, which regulates the production of a wide array of plant defensive metabolites [24,25]. However, whiteflies appear to have evolved strategies to overcome these ecological backlashes [23] and developed a strong tendency to aggregate on plant leaves in field conditions [15].

Studies show that silicon (Si) has the potential to reinforce resistance mechanisms in plants against insect herbivores with diverse feeding habits belonging to the order Homoptera [26,27], Lepidoptera [28–30], Diptera [31], Hemiptera [32,33], Coleoptera, and Thysanoptera [34] without inflicting any detrimental effects on the fitness of natural enemies [35]. Silicon also has the potential to impede the insects’ ability to suppress the defense responses of plants allowing a fully operational defense response to be instigated upon the perception of an abiotic threat [36,37]. Moreover, the potential of a particular plant species to uptake and translocate Si within its tissues varies considerably among crop species and even between varieties of the same crop species. Therefore, exogenous applications of plant-available Si sources at regular intervals are recommended to protect plants against herbivorous feeding [38–40].

Silicon abundance on Earth’s crust is second to oxygen, with concentrations fluctuating from 0.1–0.6 mM (milli molar). However, it is taken up by plants as $\text{H}_4\text{SiO}_4$ (silicic acid), the lone bioavailable silicon form present in soil solution [41]. Our knowledge regarding the positive impacts of augmenting plants with soluble Si forms has evolved substantially, principally in plant resistance to insect herbivory [42]. Yet, studies regarding optimal Si application methods according to the absorption and translocation potential of the cotton plants is still missing in literature. Therefore, the present experiments aimed to investigate the effect of different Si application methods on its accumulation in cotton leaves, a preferred feeding site of *B. tabaci*, and its impact on the oviposition preference and biology of *B. tabaci*.

2. Materials and Methods

2.1. Experimental Site

The study was conducted in a laboratory based at the School of Plant Sciences, the University of Arizona, Tucson, AZ, USA, under controlled environmental conditions.

2.2. Plant Material

Three seeds of a putative local non-Bt cotton landrace (*Gossypium hirsutum* L. cv Deltapine 5415) were grown in 15 cm diameter pots containing sterilized potting soil medium (peat moss: 3.8 cu. ft., vermiculite: 4 cu. ft., perlite: 28 gallons, and mortar sand: 21 gallons). The pots were arranged in a plant growth chamber maintained at $25 \pm 2^\circ \text{C}$,
70 ± 5% RH, and a 12:12 L:D period. The growing plants were given proper fertilization in the form of N:P:K 20:20:20. Six days after sowing, plants were thinned out by pulling all weak seedlings leaving one vigorously growing intact plant.

2.3. Insect Material

Adult whiteflies were collected stock-culture nurtured on cotton plants (Deltapine 5415) in an insect-proof mesh cage (60 × 60 × 60 cm) in an otherwise insect-free room maintained at 25 ± 2 °C, 70 ± 5% RH, and 12:12 L:D period. The stock-culture was formerly recognized as B. tabaci biotype B by Dr. Judith K. Brown, University of Arizona, Tucson, AZ, USA. As plants became fully developed, new plants were periodically rotated into the cages, and old plants were removed after adult B. tabaci had moved over to fresh plant material.

2.4. Silicon Application

The cotton plants were treated with two extremely fine powder (particle size 0.5–10 µm) forms of water-soluble silicon, i.e., silicon dioxide (SiO2) (Sigma-Aldrich, St. Louis, MO, USA) and potassium silicate (K2SiO3) (Sigma-Aldrich, St. Louis, MO, USA). Both Si sources were applied with two different application methods (foliar and drenching). However, the SiO2 solution was heated to 80 °C to achieve better solubility. Two different concentrations (200 and 400 mg/L) of silicon compounds were used for each application method; untreated controls did not receive any silicon treatment. Foliar silicon applications were applied using a 1-liter spray bottle by covering the base of the plants with paper towels to avoid soil treatment. However, silicon drenching treatments were applied directly to the potting soil medium near the base of the plants. Each silicon treatment was applied twice during the study course. Twelve days after seedling emergence, cotton plants were subjected to the first silicon applications; the follow-up treatments were applied after seven days.

2.5. Drying and Grinding of Plant Samples

Seven days after the second silicon applications, the leaves of all treated and untreated cotton plants were harvested, washed thoroughly with 0.2% detergent solution, rinsed twice with deionized water to remove potential contaminants, and dried on paper towels [43,44]. The leaves of cotton plants were then placed in paper bags and dried in a convection oven (65 °C; 48 h). The dried samples were ground in a Udy cyclone mill. The samples were then passed through a 20-mesh screen and later placed in snap-cap vials for re-drying (65 °C; 48 h). The dried samples were later stored in a desiccator until further use.

2.6. Sample Preparation (Tissue Oxidation Method)

Dry ground cotton plant samples (100 mg) were added to 50-mL polypropylene (PP) trace-metal free centrifuge tubes after the tubes had been thoroughly washed with 0.1 M (NaOH), rinsed twice with double distilled water and dried on paper towels. Five drops of octyl alcohol was added to each sample tube to reduce foaming. A 30% H2O2 solution (2 mL) was then added to the tubes in such a way that the walls of tubes were washed free of the sample. The hydrogen peroxide was added in small increments allowing time between additions for the reaction to proceed. If foaming became vigorous, a further one to two droplets of octyl alcohol were added to break the surface tension. The sample tubes were then tightly capped and arranged in a convection oven (95 °C; 30 min). After 30 min, the hot sample tubes were removed from the convection oven and later had 50% NaOH (4 mL) added. The gently vortexed and tightly capped tubes were again returned to the convection oven (95 °C; 4 h). After 4 h, the digested cotton sample tubes were carefully removed from the oven. To expedite the formation of monosilicic acid, 5 mM of NH4F (1 mL) was later added to each sample tube. The digested cotton leaf samples were then transferred to a 50-mL Nalgene volumetric flask. The final volume of digested cotton samples was achieved by dilution with distilled water [45].
2.7. ICP-MS Analysis

The silicon content in cotton plant samples was determined using ICP-MS as shown in equation (i). For this, 1 mL of diluted plant sample digest was transferred to PP tubes (13 mm × 100 mm). The sample digests were further diluted with 6 mL of distilled water to ensure that the digestion matrix did not cause any damage to the torch nozzle of the ICP instrument. Samples were analyzed for total Si using the Agilent 7700x ICP-MS (Santa Clara, CA, USA).

Tissue silicon concentration was calculated using following equation:

\[
Si_{\text{dry tissue}} (\text{g kg}^{-1}) = \left( \frac{R_{\text{Sam}} - R_{\text{Bal}}}{D} \times \frac{V_t}{V_a} \right) \times \frac{1}{S_{\text{wt}}} \times \left( \frac{10^6 \, \mu \text{g g}^{-1}}{10^6 \, \text{mg kg}^{-1}} \right)
\]  

(1)

where the ICP reading of the sample and reagent blank are represented by \( R_{\text{Sam}} \) and \( R_{\text{Bal}} \), respectively (µg Si mL\(^{-1}\)), \( D \) represents the final volume of sample in tubes submitted for analysis, final volume of the digest and volume of the digest used for ICP analysis is represented by \( V_t \) and \( V_a \), respectively, and the oven-dry equivalent weight of the sample digested (mg) is represented by \( S_{\text{wt}} \).

2.8. Free Choice Test for Oviposition Preference and Biology of B. tabaci

The oviposition preference and biology of B. tabaci was assessed seven days after the second Si application. The potted non-Bt cotton plants subjected to different Si treatments and the control group were randomly arranged inside insect-proof mesh cages. An aspirator was used to collect adult whiteflies in pairs (20/plant) from stock-culture. The collected whiteflies were released inside cages with free access to all Si treated and non-treated cotton plants. Whiteflies were allowed to feed and oviposit on cotton plants for seven consecutive days. After seven days, adult whiteflies were carefully removed from the caged cotton plants using an aspirator and plants were transferred to new insect free mesh cages. All the eggs deposited on the abaxial side of the third fully-matured apical plant leaf was counted using a microscope. For determining developmental duration of all immature stages and total cycle of B. tabaci, ten eggs were marked from each selected plant leaf \[46,47\]. The developmental period of each nymphal instar and total cycle of B. tabaci was carefully examined under a microscope.

The experimental trials involving three different factors, i.e., Si application methods (M), Si sources (S), and Si concentrations (C) were laid out in a completely randomized design (CRD). The whole experiment was repeated thrice with three non-Bt cotton plants subjected to a single treatment, serving as a replication.

2.9. Statistical Analysis

The collected data regarding silicon accumulation in cotton plants, oviposition preference, developmental period of B. tabaci nymphs, and its total cycle were analyzed using two-way analysis of variance (ANOVA). The statistically different experimental treatments were separated from each other using least significant difference (LSD) test at \( p \leq 0.05 \) \[48,49\]. All statistical analyses of the current experimental trials were carried out using the Statistics 8.1 software (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Silicon Accumulation

Silicon (Si) accumulation in non-Bt cotton plants significantly varied (\( p \leq 0.001 \)) among Si application methods (M), Si sources (S), and Si concentrations (C) (Table 1). A significantly higher Si contents was detected in leaves of those non-Bt cotton plants which were subjected to foliar applied Si (632.22 µg/g). However, the Si content in the case of Si drench was considerably lower (494.33 µg/g) as compared to foliar application. Similarly, silicon accumulation in cotton plants treated with SiO\(_2\) (634.94 µg/g) was significantly higher than those treated with K\(_2\)SiO\(_3\) (491.61 µg/g). Furthermore, the Si content in leaves of those
cotton plants which were exposed to a higher Si concentration (400 mg/L) (977.50 µg/g) was significantly higher as compared to its lower doses (200 mg/L) (585.92 µg/g) and untreated control (126.42 µg/g) (Table 1).

Table 1. Impact of Si application methods, sources, and concentrations on silicon accumulation (µg g⁻¹) in non-Bt cotton plants grown under controlled conditions.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Silicon Accumulation (µg g⁻¹) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si Application Methods (M)</td>
<td></td>
</tr>
<tr>
<td>Foliar</td>
<td>632.22 ± 3.39 a</td>
</tr>
<tr>
<td>Drenching</td>
<td>494.33 ± 3.21 b</td>
</tr>
<tr>
<td>Si Sources (S)</td>
<td></td>
</tr>
<tr>
<td>SiO₂</td>
<td>634.94 ± 3.36 a</td>
</tr>
<tr>
<td>K₂SiO₃</td>
<td>491.61 ± 3.17 b</td>
</tr>
<tr>
<td>Si Concentrations (C)</td>
<td></td>
</tr>
<tr>
<td>0 mg/L</td>
<td>126.42 ± 1.89 c</td>
</tr>
<tr>
<td>200 mg/L</td>
<td>585.92 ± 3.28 b</td>
</tr>
<tr>
<td>400 mg/L</td>
<td>977.50 ± 4.14 a</td>
</tr>
</tbody>
</table>

Least significant difference (M) 7.08
Least significant difference (S) 8.23
Least significant difference (C) 8.67
Least significant difference (M × S) 10.01
Least significant difference (M × C) 12.26
Least significant difference (S × C) 13.06
Least significant difference (M × S × C) 17.34

F-value (M) 1615.19 **
F-value (S) 1745.25 **
F-value (C) 20,554.60 **
F-value (M × S) 34.74 **
F-value (M × C) 515.14 **
F-value (S × C) 485.21 **
F-value (M × S × C) 330.41 **

Treatment means within a single column having different lower-case letters are significantly different at p ≤ 0.05; ** represents significance level at p ≤ 0.01 (LSD test).

All the possible interactions occurring between M × S × C had a significant effect (p ≤ 0.001) on Si accumulation in non-Bt cotton plants (Table 1). The interactive effect of M × S × C revealed that the foliar treatments of 400 mg/L of SiO₂ and K₂SiO₃ significantly differed (p ≤ 0.05) from their corresponding drenching treatments regarding silicon accumulation in non-Bt cotton plants (Figure 1). Maximum silicon accumulation (1235.00 µg/g) in cotton plants was recorded at the highest concentration (400 mg/L) of foliar-applied SiO₂, followed by K₂SiO₃ applied with the same application method and dose rate (987.30 µg/g) (Figure 1).
Maximum silicon accumulation (1235.00 µg/g) in cotton plants was recorded at the highest concentration (400 mg/L) of foliar-applied SiO$_2$, followed by K$_2$SiO$_3$ applied with the same application method and dose rate (987.30 µg/g) (Figure 1).

The oviposition preference of B. tabaci feeding on non-Bt cotton plants grown under controlled conditions was significantly influenced by M ($p = 0.01$), S ($p \leq 0.001$), and C ($p \leq 0.001$) (Table 2). In the case of Si application methods, foliar treatments (87.01 eggs) performed significantly better as compared to drenching treatments (95.25 eggs). Similarly, the oviposition preference of B. tabaci on non-Bt cotton plants treated with SiO$_2$ (86.70 eggs) was significantly lower as compared to K$_2$SiO$_3$-treated cotton plants (95.57 eggs). Moreover, a quantitative decline in the number of oviposited eggs was more pronounced on those cotton plants which were treated with the highest concentration (400 mg/L) (59.87 eggs) of silicon sources as compared to the lower dose rate (200 mg/L) (93.61 eggs) and untreated controls (119.83 eggs) (Table 2).

The results further revealed that the oviposition preference of B. tabaci was significantly ($p = 0.03$) influenced by the interaction of M × C (Table 2). The number of B. tabaci eggs significantly decreased when the highest concentration of Si (400 mg/L) was applied through foliar (51.11 eggs) compared to drenching (68.83 eggs) application methods (Figure 2).
Figure 2. Influence of M × C on oviposition preference of B. tabaci feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at p ≤ 0.05; and NS represents non-significance level (LSD test). Standard error (SE) values are represented by vertical bars. M: Si application methods and C: Si concentrations.

Table 2. Impact of Si application methods, sources, and concentrations on oviposition preference of B. tabaci feeding on non-Bt cotton plants grown under controlled conditions.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of Oviposited Eggs (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si Application Methods (M)</td>
<td></td>
</tr>
<tr>
<td>Foliar</td>
<td>87.01 ± 2.56 b</td>
</tr>
<tr>
<td>Drenching</td>
<td>95.25 ± 3.01 a</td>
</tr>
<tr>
<td>Si Sources (S)</td>
<td></td>
</tr>
<tr>
<td>SiO₂</td>
<td>86.70 ± 2.68 b</td>
</tr>
<tr>
<td>K₂SiO₃</td>
<td>95.57 ± 2.89 a</td>
</tr>
<tr>
<td>Si Concentrations (C)</td>
<td></td>
</tr>
<tr>
<td>0 mg/L</td>
<td>119.83 ± 3.26 a</td>
</tr>
<tr>
<td>200 mg/L</td>
<td>93.61 ± 2.76 b</td>
</tr>
<tr>
<td>400 mg/L</td>
<td>59.97 ± 2.12 c</td>
</tr>
<tr>
<td>Least significant difference (M)</td>
<td>6.27</td>
</tr>
<tr>
<td>Least significant difference (S)</td>
<td>7.13</td>
</tr>
<tr>
<td>Least significant difference (C)</td>
<td>7.68</td>
</tr>
<tr>
<td>Least significant difference (M × S)</td>
<td>8.87</td>
</tr>
<tr>
<td>Least significant difference (M × C)</td>
<td>10.86</td>
</tr>
<tr>
<td>Least significant difference (S × C)</td>
<td>11.27</td>
</tr>
<tr>
<td>Least significant difference (M × S × C)</td>
<td>15.36</td>
</tr>
<tr>
<td>F-value (M)</td>
<td>7.35 **</td>
</tr>
<tr>
<td>F-value (S)</td>
<td>8.52 **</td>
</tr>
<tr>
<td>F-value (C)</td>
<td>129.92 **</td>
</tr>
<tr>
<td>F-value (M × S)</td>
<td>1.11 NS</td>
</tr>
<tr>
<td>F-value (M × C)</td>
<td>4.01 *</td>
</tr>
<tr>
<td>F-value (S × C)</td>
<td>2.70 NS</td>
</tr>
<tr>
<td>F-value (M × S × C)</td>
<td>0.11 NS</td>
</tr>
</tbody>
</table>

Treatment means within a single column having different lower-case letters are significantly different at p ≤ 0.05; ** represents significance level at p ≤ 0.01; * represents significance level at p ≤ 0.05; and NS represents non-significance level (LSD test).
3.3. Developmental Period of *B. tabaci*

The developmental period of all nymphs and total cycle of *B. tabaci* feeding on non-Bt cotton plants grown under controlled conditions was significantly influenced by M (p ≤ 0.001), S (p ≤ 0.001), and C (p ≤ 0.001) (Table 3). An extended developmental period of all nymphs and total cycle of *B. tabaci* was observed on those cotton plant which were treated with foliar Si applications (12.9 days) as compared to its drenching treatments (11.8 days). The results further revealed that application of SiO$_2$ also caused a significant increase in the developmental period of all nymphs and total cycle of *B. tabaci* (12.8 days) as compared to its K$_2$SiO$_3$ treatment (11.9 days). Similarly, higher silicon concentration (400 mg/L) also delayed the developmental period of all nymphs and total cycle of *B. tabaci* (15.3 days) as compared to its lower concentration (200 mg/L) (11.6 days) and untreated control (10.2 days) (Table 3).

Table 3. Impact of Si application methods, sources, and concentrations on developmental period (days) of nymphs and total life cycle of *B. tabaci* feeding on non-Bt cotton plants grown under controlled conditions.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Developmental Period (Days) (Mean ± SE)</th>
<th>1st Instar</th>
<th>2nd Instar</th>
<th>3rd Instar</th>
<th>4th Instar</th>
<th>Pupae</th>
<th>1st Instar—Adult Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Si Application Methods (M)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foliar</td>
<td>2.52 ± 0.04 a</td>
<td>2.55 ± 0.05 a</td>
<td>2.71 ± 0.04 a</td>
<td>2.54 ± 0.07 a</td>
<td>2.58 ± 0.05 a</td>
<td>12.9 ± 0.20 a</td>
<td></td>
</tr>
<tr>
<td>Drenching</td>
<td>2.29 ± 0.03 b</td>
<td>2.31 ± 0.03 b</td>
<td>2.45 ± 0.03 b</td>
<td>2.36 ± 0.05 b</td>
<td>2.42 ± 0.04 b</td>
<td>11.8 ± 0.18 b</td>
<td></td>
</tr>
<tr>
<td><strong>Si Sources (S)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>2.48 ± 0.03 a</td>
<td>2.51 ± 0.05 a</td>
<td>2.69 ± 0.04 a</td>
<td>2.55 ± 0.06 a</td>
<td>2.57 ± 0.04 a</td>
<td>12.8 ± 0.19 a</td>
<td></td>
</tr>
<tr>
<td>K$_2$SiO$_3$</td>
<td>2.33 ± 0.02 b</td>
<td>2.35 ± 0.04 b</td>
<td>2.47 ± 0.03 b</td>
<td>2.35 ± 0.05 b</td>
<td>2.44 ± 0.05 b</td>
<td>11.9 ± 0.17 b</td>
<td></td>
</tr>
<tr>
<td><strong>Si Concentrations (C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mg/L</td>
<td>1.97 ± 0.02 c</td>
<td>1.96 ± 0.03 c</td>
<td>2.11 ± 0.02 c</td>
<td>2.02 ± 0.05 c</td>
<td>2.14 ± 0.04 c</td>
<td>10.2 ± 0.12 c</td>
<td></td>
</tr>
<tr>
<td>200 mg/L</td>
<td>2.28 ± 0.06 b</td>
<td>2.30 ± 0.05 b</td>
<td>2.41 ± 0.06 b</td>
<td>2.29 ± 0.07 b</td>
<td>2.32 ± 0.04 b</td>
<td>11.6 ± 0.16 b</td>
<td></td>
</tr>
<tr>
<td>400 mg/L</td>
<td>2.97 ± 0.05 a</td>
<td>3.03 ± 0.07 a</td>
<td>3.21 ± 0.04 a</td>
<td>3.04 ± 0.10 a</td>
<td>3.05 ± 0.06 a</td>
<td>15.3 ± 0.25 a</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (M)</td>
<td>0.10</td>
<td>0.12</td>
<td>0.10</td>
<td>0.16</td>
<td>0.10</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (S)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.11</td>
<td>0.17</td>
<td>0.11</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (C)</td>
<td>0.12</td>
<td>0.14</td>
<td>0.12</td>
<td>0.19</td>
<td>0.13</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (M × S)</td>
<td>0.14</td>
<td>0.17</td>
<td>0.14</td>
<td>0.21</td>
<td>0.15</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (S × C)</td>
<td>0.17</td>
<td>0.21</td>
<td>0.17</td>
<td>0.27</td>
<td>0.18</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (M × S × C)</td>
<td>0.20</td>
<td>0.21</td>
<td>0.19</td>
<td>0.31</td>
<td>0.19</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Least significant difference (M × S × C)</td>
<td>0.25</td>
<td>0.29</td>
<td>0.24</td>
<td>0.39</td>
<td>0.26</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>F-value (M)</td>
<td>20.77 **</td>
<td>16.88 **</td>
<td>28.68 **</td>
<td>5.36 *</td>
<td>10.06 **</td>
<td>25.65 **</td>
<td></td>
</tr>
<tr>
<td>F-value (S)</td>
<td>8.54 **</td>
<td>7.45 *</td>
<td>18.82 **</td>
<td>7.07 **</td>
<td>4.73 *</td>
<td>15.64 **</td>
<td></td>
</tr>
<tr>
<td>F-value (C)</td>
<td>142.80 **</td>
<td>115.31 **</td>
<td>178.98 **</td>
<td>63.08 **</td>
<td>113.39 **</td>
<td>207.14 **</td>
<td></td>
</tr>
<tr>
<td>F-value (M × S)</td>
<td>0.02 NS</td>
<td>0.11 NS</td>
<td>0.00 NS</td>
<td>0.43 NS</td>
<td>0.29 NS</td>
<td>0.00 NS</td>
<td></td>
</tr>
<tr>
<td>F-value (M × C)</td>
<td>5.33 NS</td>
<td>0.91 *</td>
<td>4.16 *</td>
<td>3.00 NS</td>
<td>7.40 **</td>
<td>6.35 **</td>
<td></td>
</tr>
<tr>
<td>F-value (S × C)</td>
<td>3.83 *</td>
<td>2.82 *</td>
<td>2.08 *</td>
<td>0.62 NS</td>
<td>1.75 NS</td>
<td>3.32 *</td>
<td></td>
</tr>
<tr>
<td>F-value (M × S × C)</td>
<td>0.10 NS</td>
<td>0.13 NS</td>
<td>0.05 NS</td>
<td>0.03 NS</td>
<td>0.36 NS</td>
<td>0.05 NS</td>
<td></td>
</tr>
</tbody>
</table>

Treatment means within a single column having different lower-case letters are significantly different at p ≤ 0.05; ** represents significance level at p ≤ 0.01; * represents significance level at p ≤ 0.05; and NS represents non-significance level (LSD test).

Furthermore, the interactive effect of M × S × C on the developmental period of *B. tabaci* feeding on non-Bt cotton plants revealed that, in the case of 1st nymphal instar, all the possible interactions occurring showed a non-significant effect (p > 0.05), except for Si source × Si concentration (S × C) (p = 0.03) (Table 3). The developmental period of 1st nymphal instar of *B. tabaci* was significantly longer on plants treated with either lower (200 mg/L: 2.39 days) and higher (400 mg/L: 3.11 days) concentrations of SiO$_2$ as
compared to corresponding concentrations of K$_2$SiO$_3$ (200 mg/L: 2.17 days) and (400 mg/L: 2.84 days) (Figure 3).

**Figure 3.** Influence of S × C on developmental period of 1st instar of *B. tabaci* feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at *p* ≤ 0.05. (LSD test). Standard error (SE) values are represented by vertical bars. S: Si sources and C: Si concentrations.

Similarly, the interactions between M × C (2nd: *p* = 0.02; 3rd: *p* = 0.03) and S × C (2nd: *p* = 0.03; 3rd: *p* = 0.04) also had a significant effect on the developmental period of 2nd and 3rd nymphal instars of *B. tabaci* feeding on non-Bt cotton plants (Table 3). A significantly prolonged developmental period of 2nd and 3rd nymphal instar of *B. tabaci* was observed on those cotton plants which were subjected to foliar treatments of higher Si concentration (400 mg/L) (2nd instar: 3.21 days; 3rd instar: 3.45 days) as compared to drenching applications of the same concentration (400 mg/L) (2nd instar: 2.86; 3rd instar: 2.98 days) (Figures 4 and 5). The results further revealed that higher (400 mg/L) and lower (200 mg/L) concentrations of SiO$_2$ (2nd instar: 3.14 and 2.44 days; 3rd instar: 3.35 and 2.57 days) significantly differed from its respective K$_2$SiO$_3$ treatments (2nd instar: 2.92 and 2.15 days; 3rd instar: 3.08 and 2.27 days) regarding the developmental period of 2nd and 3rd nymphal instar of *B. tabaci* (Figures 6 and 7).

Furthermore, all the possible interactions occurring between M × S × C showed a non-significant effect (*p* > 0.05) on the developmental period of 4th nymphal instar of *B. tabaci* (Table 3). However, in the case of pupal instar, only M × C showed a significant effect (*p* ≤ 0.001) (Table 3). A significantly longer developmental period of pupae was observed in the case of foliar application (3.27 days) of the highest Si concentration (400 mg/L) as compared to its respective drenching application (2.83 days) (Figure 8).

The results further showed that all the possible interactions occurring between M × S × C showed a non-significant effect (*p* > 0.05) on the developmental period of total cycle of *B. tabaci* feeding on non-Bt cotton plants except for M × C (*p* ≤ 0.001) and S × C (*p* = 0.04) which showed a significant effect (Table 3). The developmental period of total cycle of *B. tabaci* significantly differed on plants treated with foliar application (400 mg/L: 16.38 days; 200 mg/L: 12.02 days) of higher and lower Si concentrations (400 and 200 mg/L) as compared to its respective drenching treatments (400 mg/L: 14.28 days; 200 mg/L: 11.21 days) (Figure 9). Similarly, the higher and lower concentrations of SiO$_2$ (400 mg/L:
15.92 days; 200 mg/L: 12.25 days) significantly differed from its respective K$_2$SiO$_3$ treatments (400 mg/L: 14.74 days; 200 mg/L: 10.98 days) regarding the developmental period of total cycle of B. tabaci (Figure 10).

![Graph](image_url)

**Figure 4.** Influence of M × C on developmental period of 2nd instar of B. tabaci feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at $p \leq 0.05$. (LSD test). Standard error (SE) values are represented by vertical bars. M: Si application methods and C: Si concentrations.

![Graph](image_url)

**Figure 5.** Influence of M × C on developmental period of 3rd instar of B. tabaci feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at $p \leq 0.05$. (LSD test). Standard error (SE) values are represented by vertical bars. M: Si application methods and C: Si concentrations.
**Figure 6.** Influence of S × C on developmental period of 2nd instar of *B. tabaci* feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at *p* ≤ 0.05. (LSD test). Standard error (SE) values are represented by vertical bars. S: Si sources and C: Si concentrations.

**Figure 7.** Influence of S × C on developmental period of 3rd instar of *B. tabaci* feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at *p* ≤ 0.05. (LSD test). Standard error (SE) values are represented by vertical bars. S: Si sources and C: Si concentrations.
Furthermore, all the possible interactions occurring between $M \times S \times C$ showed a non-significant effect ($p > 0.05$) on the developmental period of 4th nymphal instar of $B. tabaci$ (Table 3). However, in the case of pupal instar, only $M \times C$ showed a significant effect ($p \leq 0.001$) (Table 3). A significantly longer developmental period of pupae was observed in the case of foliar application (3.27 days) of the highest Si concentration (400 mg/L) as compared to its respective drenching application (2.83 days) (Figure 8).

**Figure 8.** Influence of $M \times C$ on developmental period of pupal instar of $B. tabaci$ feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at $p \leq 0.05$. (LSD test). Standard error (SE) values are represented by vertical bars. $M$: Si application methods and $C$: Si concentrations.

The results further showed that all the possible interactions occurring between $M \times S \times C$ showed a non-significant effect ($p > 0.05$) on the developmental period of total cycle of $B. tabaci$ feeding on non-Bt cotton plants except for $M \times C$ ($p \leq 0.001$) and $S \times C$ ($p = 0.04$) which showed a significant effect (Table 3). The developmental period of total cycle of $B. tabaci$ significantly differed on plants treated with foliar application (400 mg/L: 16.38 days; 200 mg/L: 12.02 days) of higher and lower Si concentrations (400 and 200 mg/L) as compared to its respective drenching treatments (400 mg/L: 14.28 days; 200 mg/L: 11.21 days) (Figure 9). Similarly, the higher and lower concentrations of SiO$_2$ (400 mg/L: 15.92 days; 200 mg/L: 12.25 days) significantly differed from its respective K$_2$SiO$_3$ treatments (400 mg/L: 14.74 days; 200 mg/L: 10.98 days) regarding the developmental period of total cycle of $B. tabaci$ (Figure 10).

**Figure 9.** Influence of $M \times C$ on developmental period of total cycle of $B. tabaci$ feeding on non-Bt cotton plants grown under controlled conditions. Bars having different lower-case letters are significantly different at $p \leq 0.05$. (LSD test). Standard error (SE) values are represented by vertical bars. $M$: Si application methods and $C$: Si concentrations.
4. Discussion

Crop plants exercise specific defense responses to protect themselves from herbivorous attack [50]. However, whiteflies have evolved strategies to suppress plant defense responses by developing a strong tendency to aggregate on crop plants [14,23]. Silicon has the potential to disengage midgut epithelial cells of their target pests from its basement membrane, thus adversely affecting their efficiency of food digestion and insecticide detoxification [51,52]. Moreover, Si also rehabilitates suppressed plant defenses by reinforcing direct and indirect actions against insect herbivores [53,54]; hence can be successfully incorporated in different integrated pest management programs (IPM).

The current study revealed that silicon accumulation was more pronounced in those plants which were subjected to foliar application of SiO$_2$ (1253 µg/g) and K$_2$SiO$_3$ (987.30 µg/g) as compared to their drenching applications (SiO$_2$: 889.00 µg/g; K$_2$SiO$_3$: 798.70 µg/g). Despite its high richness in the Earth’s crust (0.1–0.6 mM) [41], the tetravalent metalloid Si is not freely reachable to plants and is locked-up in soil in the form of recalcitrant silicate minerals [55,56]. For such immobilized nutrients, foliar treatments are more effective and economical as compared to drenching treatments [29,57]. The significance of foliar application is also evident from the fact that, at initial growth stages, plant roots are not fully established to absorb mineral nutrients from the soil [58]. The transportation of such mineral nutrients from application site to the whole plant body is crucial during ontogenesis of a plant. If the applied mineral nutrient cannot translocate from treated tissues to emerging ones, spray application needs to be periodically repeated when a new flush of leaves appears on plants [59]. Silicon can translocate within the plant body through transpiration current, forming silica bodies called phytoliths [60]. However, once deposited, it becomes immovable and cannot be transported to the newly emerged leaves; hence, a continuous supply of silicon is recommended in cotton plants, keeping in mind the indeterminate growth habit of the cotton plant [38,39].

Similar findings were reported by [61], where foliar treatments of K$_2$SiO$_3$ (300 mg L$^{-1}$) significantly improved the Si contents of pepper leaves (up to 2-fold) as compared to its
respective drenching treatment. However, Si applied directly to the soil was not able to translocate the same Si content in pepper leaves as found in the roots [61]. The current study further showed that Si concentration in cotton leaves was significantly improved in those cotton plants which were exposed to higher Si concentration (400 mg/L, 977.50 µg/g) as compared to its lower dose rate (200 mg/L, 585.92 µg/g). These results are also in line with the outcomes of [62], who reported that poinsettia plants treated with a lower Si concentration (50 mg/L) accumulated less silicon content in their upper portion (<850 mg/kg) as compared to that from higher silicon treatments (100, 400, and 800 mg/L, >1100 mg/kg). Similarly, [63] demonstrated that K₂SiO₂ treatment (2 mM) significantly increased the silicon content of *Zinnia elegans* plants (12.4 g/kg) as compared to untreated controls (2.2 g/kg). The concentration of plant-assimilated silicon varies from 0.1 to 10%, depending upon crop species and absorption mechanisms [64]. Silicon absorption and translocation within plant tissues from roots to the upper plant parts is primarily regulated by numerous transporter genes (LSi1, LSi2, and LSi6) [65]. These transporter genes have already been identified in different higher plants [66,67] such as maize, pumpkin, wheat, barley, and rice [68–71]. The absence of such transporter genes in cotton plants might be the prime reason for low Si transportation from roots to upper plant tissues; further advocating the importance of Si foliar applications for controlling populations of different sap-sucking insects on cotton.

A noteworthy reduction in oviposition and increase in the developmental period of total cycle of *B. tabaci* was detected on those cotton plants which were treated with a higher Si concentration (400 mg/L: 59.97 eggs and 15.3 days) and foliar application method (87.01 eggs and 12.9 days) as compared to its respective lower concentration (200 mg/L: 93.61 eggs and 11.6 days) and application method (soil-applied: 95.25 eggs and 11.8 days), respectively. Similarly, a decline in oviposition and an increase in the developmental duration of whitefly was more pronounced on plants treated with SiO₂ (86.70 eggs and 12.8 days) than with K₂SiO₃ (95.57 eggs and 11.9 days). The current decline in the number of oviposited eggs and increase in developmental duration of total cycle of *B. tabaci* indicate that future pest population progeny generation and associated yield losses during a single crop season can be avoided to a great extent with Si applications [26].

The applied silicon accumulated in different aboveground plant tissues, primarily the cell wall epidermal, as hydrated silica (SiO₂·nH₂O) and was ultimately converted into solid-phase phytoliths [72]. These were subsequently reprocessed in the soil with the decay of the plant and again became available for new growing plants in subsequent seasons [73]. The absorbed Si makes the leaf surface harder and abrasive; consequently, wearing herbivore mandibles which reduces their food intake and biotic potential [74–77] by altering the digestibility and palatability of food material [76,78,79]. Silicon also induced certain biochemical changes in plants which initiated the production of different compounds involved in plant defenses such as momilactones, phenolics, and phytoalexins [80,81], resulting in disruption of herbivore feeding and development, making them prone to natural predation for a longer timeframe [82–84]. Moreover, production of different herbivore-induced plant volatiles is also triggered by Si application which influences the population of natural predators or parasitoids, and regulates the pest population in field conditions [34].

The current findings are in agreement with the outcomes of [32], who reported that foliar application of Si significantly deter the ovipositional preference (223.1 eggs) and increased the developmental duration (24.8 days) of *B. tabaci* on cucumber plants as compared to its respective drenching applications (317.2 eggs and 24.3 days). Similarly, wheat plants subjected to sodium silicate treatments exhibited a relatively lower nymphal population (6.5) of *Schizaphis graminum* than untreated controls (13.1). However, adults that emerged from Si-treated plots produced 80% less offspring than those from control plants [85]. Moreover, a significant decline in the mean aphid population due to silicon applications has been reported in a score of important case studies [86–90]. Furthermore, [28] also highlighted the direct relation of Si application with a prolonged developmental duration of *C. suppressalis* in rice crops. Similarly, [91] also reported that foliar Si treatments (71.2%)
significantly reduced the viability of whitefly eggs on chrysanthemum plants as compared to its drenching applications (91.6%). The results regarding ovipositional preference are also in agreement with the findings of [63], who reported that K₂SiO₃ application (29.3 eggs) significantly reduced the oviposition of green peach aphid on zinnia crops as compared to untreated crops (38.0 eggs). Furthermore, ovipositional preference, nymphal survivability, and population growth rate of rice plant hoppers was also affected as a result of silicon application [27,92,93]. Moreover, whitefly preference to deposit eggs on the most suitable host plant also validates the current reduction in the oviposition on silico-treated cotton plants [94].

Apart from eradicating pests, Si also triggers plant defense responses against diseases [95] and improves plant growth, yield, and architecture through enhancing photosynthesis [96–99], provides mechanical strength against heavy rain, wind, and lodging [100,101], diminishes salt and mineral toxicity [102–105], helps the plant to cope with water scarcity [106,107], and enhances fertilizer-use efficiency [108].

5. Conclusions

The presented research confirms the beneficial role of silicon applied directly to the soil or through cotton leaves against *B. tabaci*. The effect of silicon through foliar application provided more promising results against the target pest as compared to soil-applied silicon. However, plant responses to foliar silicon fertilization rely on various factors such as source, concentration, solubility, pH, and deliquescence point. Therefore, the potential of new readily-soluble Si sources along with their optimal doses and application times to enhance its absorption in different plant species needs to be further investigated, both in semi-natural and field conditions.

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