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Research Article

Induced resistance to *Crinocerus sanctus* (Hemiptera: Coreidae) in cowpea through silicon application

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Abstract: Silicon (Si) is a mineral known to enhance pest resistance in plants belonging to the Poaceae family. However, studies suggest potential benefits of Si in other botanical families such as Fabaceae. Thus, our hypothesis that Si applied in the form of 94.6% silicate would increase cowpea resistance to the stink bug *Crinocerus sanctus* (Fabricius), in addition to benefiting the physiological aspects of the plant. This study evaluated the vegetative development, total chlorophyll content, yield parameters, and resistance of two cowpea varieties against *C. sanctus* when sprayed with different doses of Si. Two different doses of Si were administered: the recommended manufacturer's dose (1 g L^{-1}) and a doubled dose (2 g L^{-1}), in addition to a control group (0 g L^{-1}). Plants treated with Si exhibited lower insect density and fewer pods with signs of injury. The peak insect population was observed at the onset of the cowpea's reproductive stage. Furthermore, the chlorophyll content increased from 42 mg m^{-2} (in the control group) to 48 mg m^{-2} in Si-treated plants. While the plant height and yield parameters of cowpea remained unaffected by Si application, there was a reduction in the dry mass of the aerial parts in Si-treated crops. This study demonstrated that cowpea can accumulate Si, and its application can enhance resistance against *C. sanctus.*

Keywords: Cowpea resistance, red stink bug, silicon application, *Vigna unguiculata*, yield parameters

Introduction

Cowpea *Vigna unguiculata* (L.) Walpers is one of the most economically and socially essential legumes grown in agroforestry systems worldwide, including Latin America, Africa, and Asia (Boukar *et al*., 2019). Despite its comprehensive cultivation, cowpea often

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experiences low productivity, mainly due to pest infestations that commence at seedling emergence and escalate through the production phase (Valerio, 2013). One of the major insect pests that infest cowpea is the red stink bug, *Crinocerus sanctus* (Fabricius) (Hemiptera: Coreidae) (Marsaro Jr and Pereira, 2013). The adult and immature insects feed by extracting sap from shoots, young leaves, and pods (Schaefer and Panizzi, 2000). During the feeding process, these insects inject digestive enzymes that cause lesions and deformities in plant tissue, such as pods and grains (Fazolin *et al*., 2016). Pest control in cowpea is often achieved using chemical pesticides, frequently leading to insect resistance and control failure (Yakubu *et al*., 2012).

The low resistance index against pests has been documented in cowpea germplasm strains (Boukar *et al*., 2019). Thus, host plant resistance through mineral stimulation can protect plants against pest attacks in the field (Bala *et al*., 2018). One alternative is silicon supplementation (Si) as a promoter of plant resistance against pests (Bastos *et al*., 2015). The benefits of silicon supplementation have been reported in many crops from different plants in the Poaceae family (Pilon *et al*., 2013; Xie *et al*., 2014; Flores *et al*., 2019; Kumaraswamy *et al*., 2021). Some of these benefits include increased mechanical strength of the stem and resistance to lodging in rice *Oryza sativa* L. (Garg *et al*., 2020), increased production of carbohydrates, and enhanced resistance against pests in sugarcane *Saccharum* sp. (Santos-Cividanes *et al*., 2022), and improved photosynthetic rates in maize *Zea mays* L. (Garg *et al*., 2020). In legumes, most studies on the effects of this mineral on insect resistance or physiological and yield parameters have been focused on soybean *Glycine max* (L.) Merril (Arsenault-Labrecque *et al*., 2012; Teodoro *et al*., 2015; Hussain *et al*., 2021) and cowpea (Iaguirre-Mayoral *et al*., 2017; Zhang *et al*., 2017; Putra *et al*., 2020).

Si is naturally available only at low concentrations in the soil (in the form of silicic acid and monosilicic acid), primarily in tropical regions that undergo intense weathering processes (Flores *et al*., 2019). We hypothesized that Si applied in the form of 94.6% silicate would increase cowpea resistance to the stink bug *C. sanctus*, in addition to benefiting the physiological aspects of the plant. Thus, we aimed to evaluate the vegetative development, total chlorophyll content, yield parameters, and resistance of two cowpea varieties against *C. sanctus* when sprayed with different doses of Si.

Materials and Methods

Study area

The experimental plot was set up in the experimental area of the Federal University of Pará, Campus of Altamira, state of Pará, Brazil (03º12'12"S, 52º12'23"W). The region's climate is classified as equatorial hot and humid, with three dry months in a year. The mean annual rainfall varies between 2000 and 2500 mm (INMET, 2023).

The soil is classified as an anthroposol from various landforms (Curcio *et al*., 2004). Soil samples from the 0–20 cm layer were collected and sent to the Federal Rural University of Amazonia – Brazil soil laboratory to analyze soil chemical parameters for fertility. The area was prepared by manual weeding to remove spontaneous vegetation. Sowing was performed using traditional tillage practices with a row and plant spacing of 20 × 20 cm. The plants were irrigated daily by micro-sprinkling until field capacity was reached.

Plant varieties

Two landrace varieties of cowpea, identified by lineage classification key, were used for the experiments: variety 1 (V-1) (class branco, subclass branco liso, with indeterminate growth and semi-upright bearing) and variety 2 (V-2) (class branco, subclass fradinho, with indeterminate growth and semi-prostrate bearing) (Freire Filho, 2011). These varieties are the most widely grown in the study region as they adapt well to local environmental conditions.

Experimental design

Two areas were prepared, each divided into two plots measuring 4.8 m² (2 × 2.4 m) plots, with

120 plants per plot, spaced 20 cm apart within rows and 40 cm between rows. Two rows at the ends were excluded from the sample to reduce border effects. The treatments were established in a completely randomized design, containing six treatments (plots) and four replications (planting rows with 30 plants each).

Silicon application

Commercial agricultural silicate (Alg Sil® 94.6% SiO_2-Ophicina Organica®, Brazil) was diluted in distilled water to a final concentration of 1 g L^{-1} and 2 g L^{-1} for the treatments, whereas a concentration of 0 g L^{-1} was used as the control. Si was applied by foliar spraying with a hand sprayer. The frequency of application followed the four phases of cowpea development according to Campos *et al*. (2000): the first application in the V3 phase (first trifoliate leaf fully open), second application in the V5 phase (third trifoliate leaf fully open), the third application in the V7 phase (first leaf of the secondary branch fully open), and the fourth application in the R1 phase (emergence of the first floral buds).

Determination of Si in plant tissue

At the end of the cowpea developmental cycle, leaves from the middle third of the plants of both varieties were collected from treatments with and without Si to determine the accumulated Si content in the plant tissue. The samples were dried in a forced-air circulation oven at 65 ºC for 48 h. After drying, the leaves were ground in a Willey mill, following which 100 g of the material was weighed and sent to the Laboratório de Análise Agronômica, Ambiental e Preparo de Soluções Químicas-Fullin in Linhares, Brazil for chemical analysis.

Field evaluation of *C. sanctus*

The infestation of *C. sanctus* was assessed by visually observing the insects in the field during the reproductive phase of cowpea (the period most susceptible to attack by this pest) (Marsaro Jr and Pereira, 2013). In each treatment adult insects and immature forms were counted thrice daily (8 a.m., 12 p.m., and 6 p.m.). At 60 days after planting (DAP), the number of pods damaged by insect feeding was harvested and counted.

Total chlorophyll

The total chlorophyll content, plant height, and collar diameter were measured after all applications (around 50 days after planting (DAP)). The value of total chlorophyll content (represented by ICF units) was determined by removing a leaf from each plant in the middle third of the plant and measuring it with a digital device (ClorofiLOG ® model CFL-1030, Altamira, Brazil). The height of the plant, measured from the neck to the apical bud, was determined using a ruler graduated in centimeters (cm). The collar diameter was measured 2 cm from the soil surface using a Digimess 100.176bl 200 mm/8 digital pachymeter.

Cowpea growth and production parameters

Each plot's pods were collected and quantified in the same manner (at 60 DAP) to determine the production parameters of the cowpea. Aboveground dry matter content, root dry matter, number of pods, pod length, and hundred seed weight were evaluated. Plants were separated into root and aerial parts for dry matter determination, stored in paper bags, dried in a forced-air circulation oven at 65 ºC, and weighed after 48 h (EMBRAPA, 2009). After each treatment, pods from each plot were measured using a ruler graduated in centimeters. One hundred seeds were randomly selected and weighed on precision scales graduated in grams (g) (ISTA, 2020).

Statistical analyses

All analyses were performed using generalized linear models (GLMs). The distribution of data on the number of individuals collected of *C. sanctus* and the percentage of pods with injuries was fitted to the Poisson model, with overdispersion corrected using the quasi-Poisson model. The data for total chlorophyll content, aerial and root dry mass, and yield parameters were fitted to the Gaussian model. The Si dose and variety were included in the models as explanatory variables; when no significance was found, these were removed, reducing the model to the simplest one.

Contrast analysis using GLM ($\alpha \leq 0.05$) was used to verify differences between treatment means. All analyses were performed using the statistical software R® *v.4.0.5*(R Core Team, 2023).

Results

Field evaluation of *C. sanctus*

The number of insects differed significantly between the doses of Si in the treatments ($F_{(1,178)} = 56.01$; $p \leq 0.001$). The number of insects was lower in the treatments with Si (Fig. 1A) compared to the control (Fig. 1B) for both varieties. A change in the number of individuals collected of *C. sanctus* was observed over time and during daylight hours ($F_{(1,176)} = 9.15$; $P = 0.003$). The highest population density of *C. sanctus* occurred at the R2 stage between the 40th and 42th DAP at 8:00 a.m. and 6:00 p.m., with no differences between treatments.

The percentage of pods with injury differed significantly between the varieties (Fig. 2; F (1, 19) = 4.98; P= 0.03). However, among the Si dose treatments, there were differences only for V-1 (F (1, 20) = 8.15; P = 0.01). There were no differences in this variety between the 1 g L^{-1} and 2 g L^{-1} treatments (F (2, 9) = 9.36; P = 5.91). Thus, the lowest percentage of pods with damage was found in the treatments with Si (46%), and the highest was in the control treatment (71%).

Total chlorophyll

Significant differences were found in the total chlorophyll content among plants treated with different doses of Si (Fig. 3; $F_{(1, 22)} = 6.05$; $P = 0.02$), but not among varieties ($F_{(1, 21)} = 0.56$; $P = 0.46$). The highest mean chlorophyll content was observed in plants treated with 1 g L^{-1} or 2 g L^{-1} Si (48 ± 1.66 mg m^{-2}), with no statistically significant difference between plants treated with these doses ($F_{(1, 22)} = 3.37$; $P = 0.06$); the mean chlorophyll content for control plants was 42 ± 1.81 mg m^{-2}.

Cowpea growth and production parameters

The Si concentration did not affect plant height, collar diameter, and root dry mass. No significant differences in these variables were observed among varieties. However, significant differences in aboveground dry mass were found among plants treated with different doses of Si (Fig. 4; F (1, 22) = 16.42; P = 0.0005) and between varieties (F (1, 21) = 6.56; P = 0.018). For both varieties, the highest mean dry mass was observed for control plants. The mean aboveground dry mass for V-1 and V-2 was 127 ± 3.25 g and 143 ± 7.16 g, respectively.

The yield parameters of cowpea were not affected by Si application. The differences observed were only between varieties. Variety V-1 had the highest average number of pods (Table 1; $F_{(1, 21)} = 4.26$; $P = 0.050$) (42 ± 4.60) compared to V-2 (36 ± 10.1). The V-2 variety had larger pods ($F_{(1, 21)} = 13.66$; $P = 0.001$) and higher hundred-seed weight ($F_{(1, 21)} = 41.25$; $P \leq 0.001$) with averages of 21 ± 0.30 cm and 18 ± 0.14 g, respectively, while V-1 had pods measuring 19.6 ± 0.6 cm and a hundred-seed weight of 15.8 ± 0.3 g.

Discussion

In this study, both varieties of cowpea, V-1 and V-2, showed susceptibility to *C. sanctus*. Previous studies have suggested that genes conferring resistance in cowpea lines to sucking insects are generally ineffective (Boukar *et al*., 2019). However, we demonstrate that the establishment of *C. sanctus* in cowpea plants was affected by Si treatment, and these plants can accumulate this mineral in their dry matter. Plants that accumulate Si are characterized by having Si content exceeding 10 g kg^{-1} in their dry matter, while non-accumulators typically have Si content below 5 g kg^{-1} (Ma and Yamaji, 2006; Guerriero *et al*., 2016). In this study, we collected leaf samples from plants treated and untreated with Si, sent them to a specialized laboratory for biochemical analysis, and observed a 20% increase in Si accumulation in the treated plants. Therefore, the deposition of Si led to the hypothesis of mechanical or physical barriers to *C. sanctus* feeding on cowpea by associating with the cell wall, making the cells more rigid and preventing the insect from establishing itself, penetrating the plant and feeding (direct defense) (Alhousari and Greger, 2018). Ferreira and Moraes (2011) also found that in soybeans, the deposition of Si in plant tissues made it difficult for whiteflies (*Bemisia tabaci* biotype B (Genn.)) to feed and led to more significant insect mortality in the nymph stages. Another hypothesis is that Si is also associated with the plant's biochemical/molecular defense mechanisms (indirect defense), such as enzyme regulation, hormone signaling, and alteration of volatile mixtures (Ye *et al*., 2013).

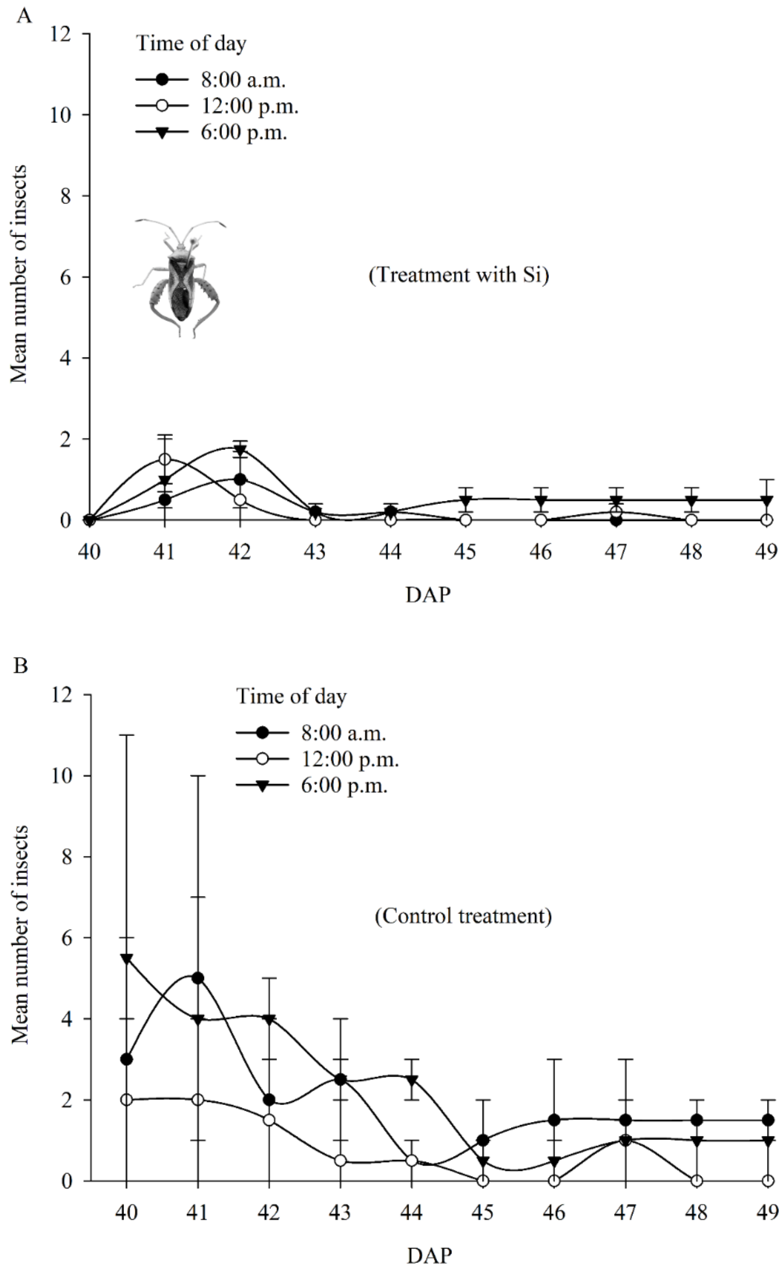

Figure 1 Mean number of *C. sanctus* during the reproductive stage of cowpea (days after planting-DAP, stage R2-R5) at different times of the day (8:00 a.m., 12:00 p.m., and 6:00 p.m.). Treatment with Si–1 g L^{-1} and 2 g L^{-1} for both varieties (Fig. A), and treatment without Si-control (Fig. B). Bars indicate confidence interval (α ≤ 0.05).

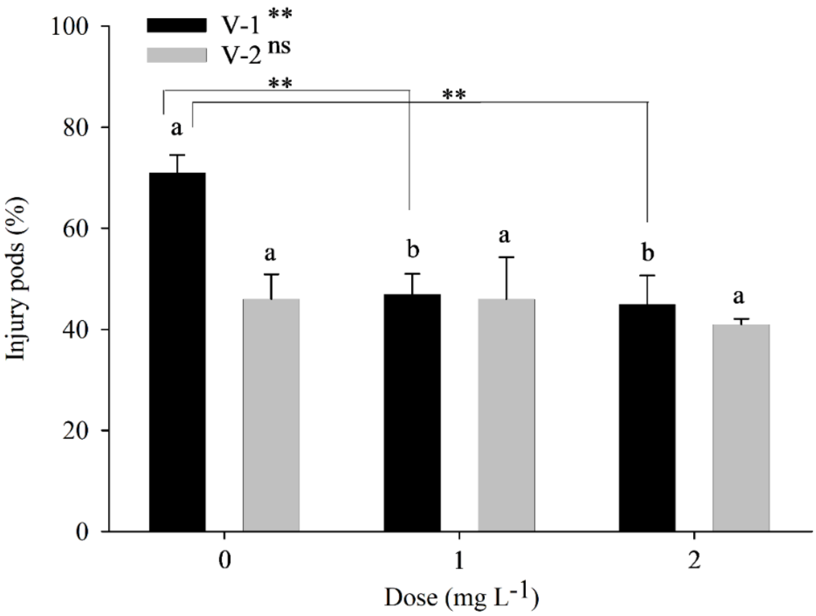

Figure 2 The proportion of pods with injury among treatments with Si dose and among varieties. Variety 1 (V-1) black bars and variety 2 (V-2) gray bars. **Indicates significant difference among Si doses for V-1 ($P < 0.05$). ns indicates no significant difference among Si doses for V-2. Lowercase letters indicate significant differences between means in treatment V-1 and capital letters for V-2, with confidence interval ($\alpha \leq 0.05$). V-1: class branco, subclass branco liso, V-2: class branco, subclass fradinho,

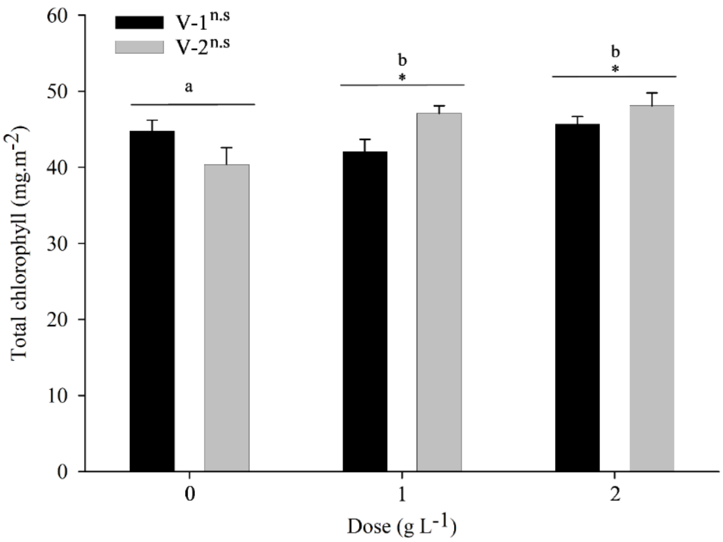

Figure 3 Total chlorophyll content among treatments with Si doses and among varieties. Variety 1 (V-1) black bars and variety 2 (V-2) gray bars. *Indicates significant difference between Si doses for V-1 ($P < 0.05$). ns indicates that there was no significant difference between Si doses for V-1 and V-2. Lowercase letters indicate significant differences between means in treatment V-1 and capital letters for V-2, with confidence interval ($\alpha \leq 0.05$). V-1: class branco, subclass branco liso, V-2: class branco, subclass fradinho,

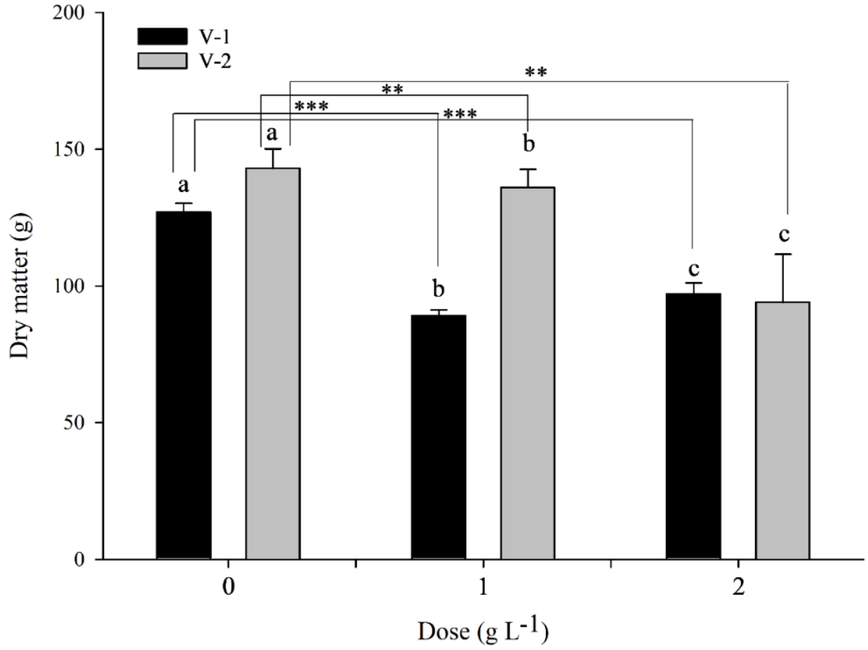

Figure 4 Mean aboveground dry mass on the treatments with Si dose and among varieties. Variety 1 (V-1) black bars and variety 2 (V-2) gray bars. ***Indicates significant difference among Si doses for V-1 ($P < 0.05$). Lowercase letters indicate significant differences between means in treatment V-1 and capital letters for V-2, with confidence interval ($\alpha \leq 0.05$). V-1: class branco, subclass branco liso, V-2: class branco, subclass fradinho,

Table 1 Yield parameters of cowpea (mean pod number, pod length, and hundred seed weight) among of Si and variety treatments (V-1 and V-2).

Entries	Dose (g L^{-1})	V-1	V-2	Anodev
Pod number	0	48 ± 3.0 Aa	33 ± 3.3 Ab	$F_{(1, 22)} = 0.15$; $P = 0.701^{ns}$
	1	37 ± 4.4 Aa	29 ± 3.1 Ab	
	2	42 ± 6.4 Aa	36 ± 10.1 Ab	
	Anodev	$F_{(1, 21)} = 4.26$; $P = 0.050^{*}$		
Pod length (cm)	0	20 ± 0.4 Aa	22 ± 0.3 Ab	$F_{(1, 21)} = 13.66$; $P = 0.461^{ns}$
	1	19 ± 0.9 Aa	21 ± 0.6 Ab	
	2	20 ± 0.5 Aa	21 ± 0.7 Ab	
	Anodev	$F_{(1, 21)} = 13.66$; $P < 0.001^{***}$		
Hundred seed weight (g)	0	16.3 ± 0.4 Aa	17.2 ± 0.1 Ab	$F_{(1, 22)} = 0.06$; $P = 0.803^{ns}$
	1	15.5 ± 0.4 Aa	17.8 ± 0.3 Ab	
	2	15.6 ± 0.3 Aa	17.7 ± 0.2 Ab	
	Anodev	$F_{(1, 21)} = 41.25$; $P < 0.001^{***}$		

[1] Means (± SD) followed by the same lowercase letters in the columns and uppercase letters in the rows among treatments do not differ statistically by contrast analysis (P < 0.05). ***Indicates significant differences among varieties. 'ns' indicates that there was no significant difference among treatments with Si doses. V-1: class branco, subclass branco liso, V-2: class branco, subclass fradinho,

The higher density of *C. sanctus* at the beginning of the reproductive stage of cowpea may be related to the feeding preference of these insects for new structures, such as floral buds and pods. During this stage, the cell wall of the substrate is typically less lignified, making it easier for the insect to penetrate the plant tissue and feed using their mouthparts, a common characteristic of sucking insects (Bussolaro *et al*., 2011). Similar results were reported by Marsaro Jr and Pereira (2013), who observed that the peak population of *C. sanctus* in beans occurred during the reproductive stage, particularly after full flowering. We also noted increased foraging activity of *C. sanctus* during the early morning and late afternoon hours. During the hottest part of the day, they may seek shelter on the abaxial side of the leaves, likely to avoid direct exposure to solar radiation and the associated thermoregulation costs.

The total chlorophyll content increased in both cowpea varieties after Si application, a trend also observed in previous studies involving soybean and cowpea (Teodoro *et al*., 2015; Pereira *et al*., 2017). Chlorophyll plays a crucial role in the photosynthetic process of plants, as it absorbs light energy and converts it into chemical energy. This energy is then utilized in the synthesis of various compounds, including carbohydrates (Kluge *et al*., 2015). The enhanced chlorophyll production in plants promotes increased energy availability for the synthesis of compounds associated with defense mechanisms against both biotic and abiotic stresses (Taiz *et al*., 2017).

Numerous previous studies have reported enhanced growth and production parameters in various crops following Si treatments (Bussolaro *et al*., 2011; Carvalho *et al*., 2015; Teodoro *et al*., 2015; Artyszak, 2018; Alam *et al*., 2021; Oliveira *et al*., 2024). In this study, Si appeared to primarily impact the dry mass content of the plants, potentially due to the administered doses exerting a more pronounced influence on the photoassimilation pathways associated with the plant's defense mechanisms rather than on agronomic characteristics (Heil and Bostock, 2002). Nonetheless, further investigations are warranted to ascertain whether lower Si doses affect growth and production mechanisms in this crop, as Si application exceeding 100 mg/L may induce the formation of a non-absorbable gelatinous web of compounds linked to plant growth and production (Prado *et al*., 2019).

Conclusion

This study represents the first to document the resistance conferred by Si in cowpea against the stink bug *C. sanctus*. Furthermore, cowpea can accumulate this element in their leaves, positively impacting total chlorophyll content. Silicon is a naturally occurring element that is non-toxic to humans and beneficial insects. Its utilization in agriculture holds promise as an alternative for the control of *C. sanctus* and other sucking insects. Subsequent research is essential to explore alternative Si sources like calcium and potassium silicates and assess their implications for legume development, productivity, and insect and disease control.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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مقاومت القایی به سن *Crinocerus sanctus* (Hemiptera: Coreidae) در لوبیا چشم بلبلی از طریق کاربرد سیلیس

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چکیده: سیلیس (Si) یک ماده معدنی شناخته شده برای افزایش مقاومت به آفات در گیاهان متعلق به تیره Poaceae است. با این حال، مطالعات مزایای بالقوه Si را در سایر تیره‌های گیاهی مانند Fabaceae نشان می دهد. بنابراین، فرضیه ما مبنی بر استفاده از Si به‌شکل۹۴/۶ درصد سیلیکات، مقاومت لوبیا چشم‌بلبلی را در برابر حشره *Crinocerus sanctus* (Fabricius) افزایش می‌دهد، علاوه‌براین‌که از جنبه‌های فیزیولوژیکی گیاه نیز سود می‌برد. این مطالعه رشد رویشی، محتوای کلروفیل کل، پارامترهای عملکرد و مقاومت دو رقم لوبیا چشم‌بلبلی در برابر *C. sanctus* را هنگامی که با دزهای مختلف Si اسپری می‌شوند، ارزیابی کرد. دو دز مختلف Si تجویز شد: دز توصیه شده سازنده (۱ گرم در لیتر) و دز دو برابر شده (۲ گرم در لیتر)، علاوه بر گروه کنترل (۰ گرم در لیتر). گیاهان تیمار شده با Si تراکم حشره کم‌تر و غلاف‌های کم‌تری با علائم خسارت نشان دادند. اوج جمعیت حشرات در شروع مرحله زایشی لوبیا چشم‌بلبلی مشاهده شد. علاوه‌براین، محتوای کلروفیل از ۴۲ میلی‌گرم در مترمربع (در گروه شاهد) به ۴۸ میلی‌گرم در مترمربع در گیاهان تیمار شده با سیلیس افزایش یافت. درحالی‌که ارتفاع گیاه و پارامترهای عملکرد لوبیا چشم‌بلبلی تحت تأثیر کاربرد سیلیس باقی ماندند، کاهش در توده خشک اندام هوایی در محصولات تیمار شده با سیلیس وجود داشت. این مطالعه نشان داد که لوبیا چشم‌بلبلی می‌تواند سیلیس تجمع کند و کاربرد آن می‌تواند مقاومت در برابر *C. sanctus* را افزایش دهد.

واژگان کلیدی: مقاومت به لوبیا چشم‌بلبلی، *Crinocerus sanctus*، کاربرد سیلیس، *Vigna unguiculata*، پارامترهای عملکرد