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Silicon as resistance inducer to control black apphid *Aphis craccivora* Koch, 1854 in cowpea beans [*Vigna unguiculata* (L.)] Walp.

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ABSTRACT
The black aphid *Aphis craccivora* (Koch, 1854) stands out between the bugs considered cowpea pests. The objective of this study was to evaluate the effects of silicon application on the resistance induction of cowpea plants to the black aphid *A. craccivora*. The experiment was conducted in the Entomology Laboratory of the Phytosanitary sector of the Centro de Ciências Agrárias at the Universidade Federal do Piauí. The effects of the application of silicon on biological aspects were evaluated using a completely randomized design, with four treatments and 40 repetitions, being: silicon in soil (T1), silicon in soil + leaf (T2), silicone leaf (T3), and control (T4). The following biological variables were evaluated: generation period, reproductive period, fecundity, and daily average of nymphs per female. The silicon and lignin contents were also evaluated in the plants. The silicic acid was applied in a 1% solution around the stem of the plants (soil), 15 days after emergence, by diluting 2 g of the product in 200 mL of water. However, the leaf application was carried out with sprayer five days after application in soil. The non-preference of *A. craccivora* on bean was also evaluated. The evaluations were performed after 24, 48 and 72 hours of infestation by counting nymphs at 24, 48 and 72 hours and adults at each leaf session. The application of silicon promotes the reduction of the production of nymphs, interfering in the biological aspects of *A. craccivora*, and has potential to be used in a cowpea pest management program in cowpea.

Keywords: plant protection; antibiosis; sucking insects; integrated pest management; elicitor.

INTRODUCTION

Cowpea, cowpea or macassar bean [*Vigna unguiculata* (L.) Walp.] have their origins on the African continent and were introduced to Brazil in the 17th century, becoming an important agricultural crop in the country, especially in the northern and northeast regions (FREIRE FILHO et al., 2011).

According to FREIRE FILHO et al. (2011), the crop is quite rustic, adapts to different soil conditions, consumes little water, and has a rapid cycle. Cowpea constitutes one of the main social and economic alternatives for food supply for the low-income population. The culture has greater economic importance in the north and northeast, but is expanding outside this axis, mainly to the cerrado of the central west.

There are several insects associated with cowpea cultivation, among which the aphid *Aphis craccivora* stands out, considered one of the main pests, occurring in Africa, Asia and Latin America (DE LA PAVAS; SEPÚLVEDA-CANO, 2015).

Aphis craccivora is a cosmopolitan and polyphagous insect. It receives different names such as black aphid, black bean aphid, black legume aphid, cowpea aphid, among others (SINGH; SINGH, 2017).

Control is basically done using chemical control, but there are other defense strategies, such as induced defenses. Plants have developed over time several defense mechanisms against herbivory caused by insects. These defense mechanisms

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are divided into constitutive or direct defenses, specific to the species, and induced or indirect defenses, common to plants in general (FERNANDES et al., 2009).

Induced defenses appear after herbivory. This defense mechanism begins with contact between insects and plants, going through the elicitation of signals and biosynthesis of several compounds involved in defense. These mechanisms are complex and are not yet completely understood (ARIMURA et al., 2005).

Silicon is an element present in plants, and the amount varies from one species to another, and can act directly in reducing damage caused by insects. The mechanisms by which silicon can increase resistance to insects refer to increased physical resistance through greater deposition of amorphous silica, leading to greater hardness of epidermal tissues, lower digestibility, and the production of defensive enzymes or possibly the release of volatiles by plants. In addition to these effects, others have already been determined, such as the reduction in insect growth and reproduction (KEEPING et al., 2009; REYNOLDS et al., 2009; ALCANTRA et al., 2010).

Silicon is recognized as an inducer of plant defense against insect attack, both against sucking (ALMEIDA et al., 2015; DALASTRA et al., 2011; FILGUEIRAS et al., 2011) and chewing insects (KEEPING et al., 2009; ANTUNES et al., 2010).

In view of the above, the objective was to evaluate the effect of silicon as a resistance inducer in cowpea *V. unguiculata* in relation to attack by the black aphid *A. craccivora*.

MATERIAL AND METHODS

The experiment was conducted at the Centro de Ciências Agrárias at the Universidade Federal do Piauí, located in the municipality of Teresina, Piauí, Brazil, with geographic coordinates 05°05'21"S and 42°48'07"W, sea level height of 72 m, average maximum and minimum temperatures of 34 and 22°C, respectively, average maximum and minimum humidity of 84 and 56% (ANDRADE JÚNIOR et al., 2004).

The research was carried out using a completely randomized design, with four treatments:

- Treatment 1: silicon in the soil;
- Treatment 2: silicon in the soil + silicon in the leaves;
- Treatment 3: leaf silicon;
- Treatment 4: control.

Silicon was supplied in the form of 1% silicic acid solution. Silicic acid was applied around the plant stem (soil), 15 days after emergence, diluting 2 g of the product in 200 mL of water. Foliar application was carried out with a 1-L sprayer five days after application in a drench adapted from the methodology by ANTUNES et al. (2010).

In a greenhouse, cowpea plants were grown in 2.8-L pots placed on benches, containing a mixture of soil, cured farmyard manure and sand (3:1:1). Seeds from the BRS Imponente cultivar, chosen in a pre-test carried out, were used. The Imponente genotype bean seeds were provided by the Germplasm Bank of the Agricultural Research Company Embrapa Meio Norte. Cowpea plants were cultivated, planting three seeds per pot and subsequently thinning, leaving only one more vigorous plant per pot. The plants were watered daily every day of the experiment. The aphids used were the same age and came from farms, kept in a greenhouse, and fed on broad bean plants.

For the free choice test, 2 × 2-cm bean leaf sections from the four treatments were used, arranged in 15-cm diameter petri dishes, with the bottom covered with sponge and water. The four sections were randomly distributed and equidistant from each other, allowing free choice by the aphids. Twenty-five adult apterous aphids were released in the center of each plate 24 hours after application of all treatments. The plates were placed in a biochemical oxygen demand chamber regulated with a temperature of 25 ± 2°C, relative humidity of 70% and a 12-hour photophase. After 24, 48 and 72 h of infestation with aphids, live nymphs were counted (removed immediately after counting, with these values being accumulated in the following counts), as well as adults present in each leaf section.

In the no-choice test, a leaf section, 2 × 2-cm, from each treatment was placed in 5-cm diameter petri dishes, with the bottom covered with sponge and water. One adult was released on each plate, and, 24 hours after placement, a nymph was removed and selected, which remained on the plate until its death.

The biological variables observed were: period of one generation; reproductive period; fecundity; and the daily average of nymphs per female, determining the lignin and silicon contents in each treatment.

The data were subjected to analysis of variance, using the Bioestat computer program (AYRES et al., 2007), version 5.0, of 2007.

The determination of silicon content was carried out in the soil laboratory belonging to the Soil Department of the Agricultural Sciences Center of the Universidade Federal de Viçosa, MG, Brazil. Extraction was done with 3/1 nitric and perchloric acid. After extracting the sample, it was read using an Agilent model 240FS spectrophotometer.

The determination of lignin content was carried out at the Animal Nutrition Laboratory belonging to the Animal Husbandry Department of the Agricultural Sciences Center at the Universidade Federal do Piauí. Lignin was determined using acid detergent fiber, and the 72% sulfuric acid method, as recommended by SILVA (1981).

RESULTS AND DISCUSSION

Regarding the preference test with chances of choice, no significant differences were observed between treatments in relation to the number of adults and nymphs (Table 1). From the results, it can be seen that silicon did not have a repellent effect on adults, and with regard to nymphs, even though we have noticed a greater tendency in the control at 48 and 72 hours, it was not statistically significant. The explanation is probably that silicon needs more time to activate the plant's defenses. Non-preference leads the insect to not feed normally on the plant.

Table 1. Number of adults, nymphs at 24 hours and number of accumulated nymphs at 48 and 72 hours of *Aphis craccivora*, in a test with choice, in cowpea.

Treatments	Adults			Nymphs		
	24 hours^{ns}	48 hours^{ns}	72 hours^{ns}	24 hours^{ns}	48 hours^{ns}	72 hours^{ns}
Silicon in soil	3.40 ± 0.80	12.20 ± 0.86	0.70 ± 0.33	14.50 ± 3.37	28.80 ± 8.73	32.20 ± 9.12
Soil + leaf silicon	3.60 ± 0.84	2.23 ± 0.74	1.70 ± 0.78	12.10 ± 3.45	27.20 ± 8.17	34.20 ± 7.55
Leaf silicon	2.00 ± 0.44	2.80 ± 0.55	0.55 ± 0.51	7.90 ± 2.50	26.40 ± 6.37	30.40 ± 6.05
Control	2.16 ± 0.68	2.30 ± 0.51	2.20 ± 0.67	13.00 ± 2.98	34.30 ± 3.59	45.40 ± 9.69
Test F	F = 10.42	F = 0.6417	F=11.42	F = 0.8311	F = 0.2577	F = 0.8695
p-value	(p = 0.3865)	(p = 0.5967)	(p = 0.3456)	(p = 0.5119)	(p = 0.8563)	(p = 0.5318)

ns: not significant at the 5% level by the test F.
Source: Elaborated by the authors.

PEIXOTO et al. (2011), in a test with choice, state that, in bean plants in which silicon was applied, the plant induced non-preferences for oviposition and affected the development of whitefly nymphs *Bemisia tabaci* biotype B. NASCIMENTO et al. (2014), researching induced resistance in rice to *Spodoptera frugiperda* by the application of silicon, in a test with choice, observed that there was no significant difference ($p > 0.05$) between the treatments in relation to the food preference of the caterpillars in the first 24 hours. At 48 and 72 hours, plants treated with silicon showed greater protection against the insect pest.

In the no-choice test, biological variables were analyzed: period of one generation, reproductive period, fecundity, and daily average of nymphs per female (Table 2). Among the biological parameters of the black aphid evaluated after application of silicon, there were no significant differences in the pre-reproductive period between the treatments in which silicon was applied. There were significant differences between leaf silicon and control. Leaf silicon showed the shortest nymphal period. Perhaps some substance induced by silicon, such as a greater amount of lignin, in the foliar application, reduced this period.

Table 2. Biological aspects (nymphal period, longevity, fecundity, and daily average of nymphs per female) of *Aphis craccivora* aphids on cowpea plants treated with silicon (mean ± standard error).

Treatments	Nymphal period	Longevity ^{ns}	Fecundity	DAN/female
Silicon in soil	4.32 ± 0.08 ab	17.32 ± 0.20	75.95 ± 3.81 b	5.79 ± 0.24 b
Soil + leaf silicon	4.17 ± 0.06 ab	17.60 ± 0.31	76.42 ± 3.31 b	5.73 ± 0.24 b
Leaf silicon	4.07 ± 0.04 b	18.05 ± 0.26	86.42 ± 3.74 ab	6.19 ± 0.24 ab
Control	4.35 ± 0.08 a	17.03 ± 0.24	90.07 ± 2.69 a	6.97 ± 0.18 a
Test F	3.97	2.49	4.34	6.21
P	0.0094	4.96	0.0060	0.0008

ns: not significant; DAN/female: daily average number of nymphs per female.
Source: Elaborated by the authors.

Likewise, there were no significant differences between the different treatments regarding longevity (Table 2), showing that this variable was not affected by silicon. Even though silicon was in greater quantity in the plant in treatments that received silicon, the element did not change the longevity of the insects. COSTA and CAMPOS (2006) researched the use of silicon on the biology of the green aphid on wheat and observed that silicon did not affect the pre-reproductive period either. GOMES (2008) verified the use of silicon as an inducer of resistance to *Myzus persicae* and observed that silicon did not affect the pre-reproductive period and longevity.

Fecundity and the daily average of nymphs (Table 2) were affected by the application of silicon. Plants that did not receive silicon had a greater number of nymphs, and the best treatment was in which half the dose of silicon was applied to the soil and the other half to the leaves. It was an important result of plant resistance to insects, as less nymph production results in less damage. Resistance in the present study was probably due to antibiosis, in which one of the characteristics is the decrease in the number of nymphs. The nutritional quality of the plant interferes with the fecundity of the insect. It was assumed that silicon leads to greater hardening of the cell wall, making it difficult for insects to feed and that it induces the plant to produce substances with deterrent characteristics, which hinder the absorption of essential nutrients.

According to KORNDÖRFER et al. (2011), resistance is not related to the amount of silicon, but to how and where it is distributed and organized in the plant. The nutritional quality of the host plant can affect fecundity.

ANTUNES et al. (2010), studying the influence of silicon on the occurrence of *Spodoptera frugiperda*, state that silicon does not directly affect the insect, but favors the predator *Dorus spp.*, an important predator of the caterpillar. In sunflowers, silicon reduces the infestation of the sunflower caterpillar *Chlosyne lacinia*. DALASTRA et al. (2011) state that the application of silicon provides protection to the plant by reducing the number of silver thrips adults and nymphs in peanuts. A similar result was obtained by FILGUEIRAS et al. (2011) studying the application of silicon in the induction of the green wheat aphid *Schizaphis graminum*. PORTELA et al. (2019), researching the effect of silicon on the aphid *A. craccivora* in the fava bean crop *Phaseolus lunatus*, concluded that the insect's fecundity is affected by silicon, a result similar to the present study.

Figure 1 shows the lignin content in the leaves and stems of cowpea, showing a greater accumulation in the treatment in which the silicon was applied half the dose to the soil and half to the leaves, not statistically different from the treatment in which all the silicon was applied to the soil. It is assumed that lignin interfered with the reduction in the fecundity of aphid females, as treatments in which there was greater lignin accumulation had also lower fecundity.

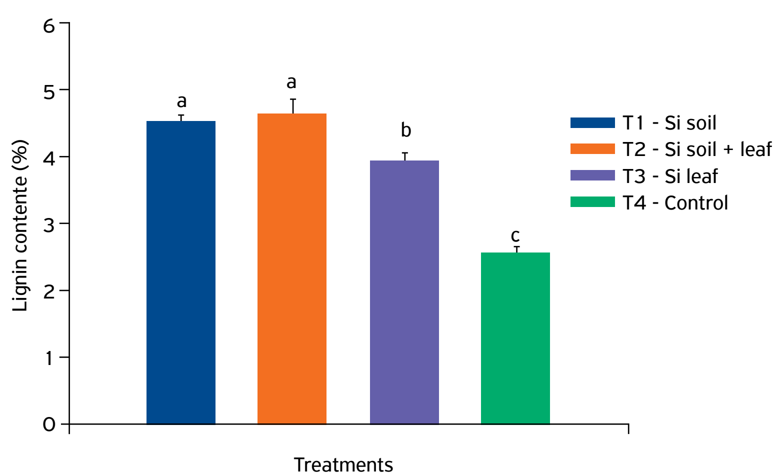

Figura 1. Lignin content (% and standard error) in dry mass of the aerial part (leaves and stems) of cowpea in different treatments.
Source: Elaborated by the authors.

According to SANTIAGO et al. (2013), tissue hardness is one of the key factors that regulate herbivore damage to plants. High lignin concentrations imply a less palatable food for insects, and the greater tenacity and concentrations of toxic byproducts of lignin synthesis appear to be the most likely mechanisms for lignin-associated protection of plant tissues against insects and disease. According to FREI (2013), insects do not have the ability to decompose lignin. GOMES et al. (2005) and OLIVEIRA et al. (2012) state that silicon plays an important role by stimulating the production of enzymes related to defense against herbivores. The production of peroxidase, for example, is related to a greater deposition of lignin and suberin, substances that increase tissue hardness and reduce the nutritional quality of food for herbivores.

The silicon content in the leaves and stem was also analyzed, as shown in Fig. 2. Despite the large difference between the different treatments, there were no significant differences, due to the large dispersion of the data. Although there are no differences between treatments, the figures for lignin and silicon contents are very similar, probably showing the beneficial effect of silicon in increasing the lignin content of plants.

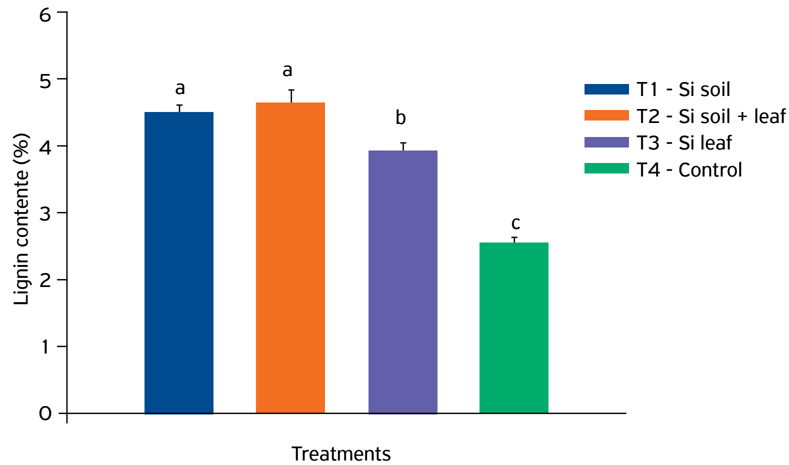

Figura 2. Silicon content (mg/kg +standard error) in dry mass of the aerial part (leaves and stems) of cowpea in different treatments.
Source: Elaborated by the authors.

According to MALI and AERY (2009), plants are classified as silicon accumulators and non-accumulators, and, even though cowpea is in the group of non-accumulator plants, several studies have demonstrated that silicon is beneficial for the crop, especially when applied to the soil. According to KORNDÖRFER et al. (2011), silicon deposition is capable of creating resistance against some insects, due to physical, chemical and structural barriers. The use of silicon in cowpea cultivation has the potential to be used in a cowpea pest management program.

CONCLUSION

The application of silicon interferes with the biology of *A. craccivora*, mainly causing reduction in the production of nymphs.

AUTHORS' CONTRIBUTIONS

Conceptualization: Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M.; Girão Filho, J.E. **Formal analysis:** Portela, G.L.F. **Investigation:** Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M.; Girão Filho, J.E. **Methodology:** Portela, G.L.F.; Silva, P.R.R. **Project administration:** Portela, G.L.F. **Resources:** Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M.; Girão Filho, J.E. **Software:** Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M.; Girão Filho, J.E. **Supervision:** Portela, G.L.F.; Silva, P.R.R. **Visualization:** Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M. **Writing – original draft:** Portela, G.L.F.; Silva, P.R.R.; Pádua, L.E. de M.; Girão Filho, J.E. **Writing – review & editing:** Portela, G.L.F.; Silva, P.R.R.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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