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Control of *Meloidogyne javanica* in cowpea with silicon application

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ABSTRACT: Cowpea (*Vigna unguiculata* (L) Walp) can suffer significant losses due to the attack of *Meloidogyne javanica*. The most effective control is through nematicides, however, it is an expensive treatment, so alternative measures have been sought. Therefore, the objective was to evaluate the effect of silicon on the control of *M. javanica* in cowpea plants. The experiment was carried out in a greenhouse, in a completely randomized design, in a 5 × 2 + 1 factorial scheme, being: five doses of Si (0, 60, 120, 180, and 240 mg dm^{-3}), two soluble sources of Si (Quimifol Silício® and sodium silicate P.A.), plus an additional treatment consisting of tomato cultivation in soil without the addition of Si and with nematodes, with four replications. Sixty days after inoculation in the plants, the root systems were collected and evaluated: volume, length and fresh mass of roots, gall index, egg mass index, reproduction factor, reproduction index, and reduction of reproductive factor of *M. javanica*. The sources of silicon at different doses had a positive effect on root variables. They were also able to reduce the parasitism variables of *M. javanica* in cowpea plants.

Key words: alternative control; root-knot nematodes; sodium silicate; *Vigna unguiculata*

Controle de *Meloidogyne javanica* em feijão-caupi com aplicação de silício

RESUMO: O cultura do feijão-caupi (*Vigna unguiculata* (L) Walp) pode sofrer perdas significativas devido ao ataque de *Meloidogyne javanica*. O controle mais efetivo é através de nematicidas, no entanto, é um tratamento caro, assim tem-se buscado medidas alternativas. Logo, objetivou-se avaliar o efeito do silício no controle de *M. javanica* em plantas de feijão-caupi. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, em esquema fatorial 5 × 2 + 1, sendo: cinco doses de Si (0, 60, 120, 180 e 240 mg dm^{-3}), duas fontes solúveis de Si (Quimifol Silício® e silicato de sódio P.A.), mais um tratamento adicional constituído pelo cultivo do tomateiro em solo sem adição de Si e com nematoides, com quatro repetições. Sessenta dias após inoculação nas plantas, os sistemas radiculares foram coletados e avaliado: volume, comprimento e massa fresca das raízes, índice de galhas, índice de massa de ovos, fator de reprodução, índice de reprodução e redução do fator reprodutivo de *M. javanica*. As fontes de silício nas diferentes doses tiveram efeito positivo para as variáveis radiculares. Também foram capazes de reduzir as variáveis de parasitismo de *M. javanica* em plantas de feijão-caupi.

Palavras-chave: controle alternativo; nematoides das galhas; silicato de sódio; *Vigna unguiculata*

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Introduction

Cowpea (*Vigna unguiculata* (L) Walp) is an annual legume, of African origin, with wide adaptation to water, heat and salt stresses, grown mainly in the dry tropical areas of Africa, Latin America, and South Asia (Boukar et al., 2018; Panchta et al., 2021). Its grains are widely used in human food due to being a rich source of protein, carbohydrates, as well as presenting other components such as fiber, iron, calcium, zinc, among others (Gondwe et al., 2019).

Like other crops, it is subject to significant losses in productivity due to biotic and abiotic factors, as well as management (Kebede & Bekeko, 2020). Continuous cultivation linked to monoculture, as is the case with cowpea in many regions, impairs soil quality, also resulting in the accumulation of plant parasitic nematodes (Enyiukwu et al., 2021). Among the species of nematodes that parasitize the crop, there is *Meloidogyne javanica*, a species considered economically important because it has a wide range of hosts, a high capacity for dissemination and high aggressiveness, in addition to the low level of resistance of cultivated materials, resulting in significant losses (Khanal et al., 2016; Su et al., 2017; Chi et al., 2020; Alonso et al., 2022).

Although chemical nematicides have been used as one of the main means of nematode control because of their fast and efficient results, their use is associated with high costs, and this is a crop grown mainly by small and medium producers, which sometimes makes their use unfeasible, and can also have negative effects on human health and the environment (Hemmati & Saeedizadeh, 2020; Enyiukwu et al., 2021). Thus, alternative measures have been sought.

In recent years, the management measures for plant diseases have been more appealing for preventive intervention, aiming to reduce costs. Among the alternatives, the recommendation of silicon (Si), although not an essential element for plants, has shown promising results in controlling nematodes in different crops (Rajput et al., 2021; Santos et al., 2021; Alonso et al., 2022). Silicon induced defense mechanisms include: accumulation of lignin, phenolic compounds, increased ascorbic acid concentration, and activation of resistance-related enzymes and phytoalexins, among others (Guerriero et al., 2016; Alonso et al., 2022).

Despite being widely studied in other crops, the effects of silicon on the relationship of cowpea plants and *M. javanica* are scarce. Thus, the objective was to evaluate the effect of silicon on the control of *M. javanica* in cowpea plants.

Materials and Methods

Location and characterization of the experimental area

The experiment was conducted in a greenhouse and in the Laboratory of Plant Pathology, both located in the Center of Agro-Food Sciences and Technology of the Universidade Federal de Campina Grande - CCTA/UFCG, Pombal Campus, PB, Brazil.

The experiment was conducted with samples of chromic Luvissolo (Embrapa, 2006), with absent Si content, randomly collected in the 0 to 0.30 m depth layer, with the following soil characteristics: for the chemical attributes – pH (CaCL) = 6.03; H + Al = 0.50; Al^{3+} = 0.50; P = 9.17 mg kg^{-1}; K^+ = 0.40 cmol dm^{-3}; Na^+ = 1.04 cmol dm^{-3}; Ca^{2+} = 4.8 cmol dm^{-3}; Mg^{2+} = 3.30 cmol dm^{-3}; OM = 7.40 g kg^{-1}; and CTC^+ = 9.54 cmol dm^{-3}; for the physical characteristics: sand = 636.8 g kg^{-1}; silt = 97.2 g kg^{-1}; clay = 266.0 g kg^{-1}; Ds = 1.36 g cm^{-3}; Dp = 2.64 g cm^{-3}; and porosity = 0.48 m^3 m^{-3}, being classified with Sandy Loam textural class.

Experimental procedures

The treatments were constituted by a 5 × 2 + 1 factorial arrangement comprising 5 doses of Si (0, 60, 120, 180, and 240 mg dm^{-3}) and two soluble sources of Si (as a commercial source of Si, the product Quimifol Silício® was used, containing 10% of Si and 8.3% of K and density of 1.31 kg L^{-1}, while the source P.A., in the form of sodium silicate, used the reagent Dynamic® containing 29.4% of Si and 13.5% of Na) and an additional treatment consisting of growing the bean on soil without adding Si and with inoculation (Table 1). The experimental design was entirely randomized, with four repetitions, totaling 44 experimental units. Each experimental unit consisted of a plastic pot containing 4 dm^{-3} of substrate with two plants. Before installation of the experiment, the soil used to fill the pots was sterilized in an autoclave at 120 °C under pressure of 1.05 Kgf cm^{-2} for 2 hours.

The fertilization with macro and micronutrients was performed according to the recommendations of Novais et al. (1991), who recommend the following doses in mg dm^{-3}: N = 100-300; P = 200-300; K = 150-200; Ca = 200; Mg = 50; S = 40-50; B = 0.5-0.8; Cu = 1.3-1.5; Fe = 2-5; Mn = 3-4; Mo = 0.10-0.15; and Zn = 4-5, for experiments in soil pots, with appropriate adjustments depending on the results of the soil analysis. When calculating the amounts of nutrient sources used in fertilization, the amounts of Na and K supplied by the sources of Si used were discounted, aiming to balance the doses of these elements among the treatments tested.

Then, the doses of silicate were added, according to the respective treatments, and the product was mixed into the

Table 1. Treatments formulated from sources and doses of silicon.

Treatments	Source of Si	Doses of Si (g L^{-1})
Water	-	0
F1-60	F1 (10% Si and 8.3% K)	60
FI-120	F1 (10% Si and 8.3% K)	120
F1-180	F1 (10% Si and 8.3% K)	180
F1-240	F1 (10% Si and 8.3% K)	240
F2-60	F2 (29.4% Si and 13.5% Na)	60
F2-120	F2 (29.4% Si and 13.5% Na)	120
F2-180	F2 (29.4% Si and 13.5% Na)	180
F2-240	F2 (29.4% Si and 13.5% Na)	240
Tomato tree	-	0

F1: Quimifol Silício®; F2: Sodium silicate.

substrate. Cowpea variety cowpea was sown directly into pots containing a substrate consisting of a mixture of sandy soil and bovine manure in a 2:1 ratio. Five days after emergence, the plants were thinned, leaving only one plant per pot. Irrigation was performed manually as needed by the crop.

Multiplication of inocula and inoculation

The inoculum of *M. javanica*, was composed of eggs and larvae, obtained from pure populations, collected from roots of tomato plants cv. Santa Cruz, kept in a greenhouse in multiplication. It was previously identified, with the aid of an optical microscope, in a morpho-anatomical study by examining the perineal configuration against the specific literature (Hartman & Sasser, 1985).

After the cotyledons of the cowpea plants had fallen off, the nematodes were extracted from the tomato roots using the Hussey & Barker (1973) technique, modified by Boneti & Ferraz (1981), and inoculated into the cowpea plants as a suspension, the equivalent of 4,100 eggs/juveniles distributed in three openings (holes) approximately 3.0 cm deep, 2.0 cm apart from each other and from the hypocotyl of the plants. The plants were irrigated twice daily to meet physiological needs and provide the pathogen/host infection relationship.

Agronomic characteristics

The evaluations were carried out sixty days after the cowpea plants were coexisting with the gall nematodes. As for the agronomic characteristics, the variables evaluated were: root volume (RV), root length (RL), and root fresh mass (RFM).

To determine the volume of roots, a 1,000 mL beaker was used, considering a fixed initial volume of 800 mL of water, the roots were immersed in this volume and thus, it was calculated between the initial volume by the final volume, obtaining then the volume of the roots. Before the procedure, the roots were placed on paper towels on a bench to remove excess water. For the root length, it was measured with a graduated ruler, where on the flat bench, the roots were fully stretched to determine their length. For root fresh mass, the roots were washed under running water to remove aggregates, then weighed on a semi-analytical balance.

Evaluation of parasitism on plants

The following parasitism characteristics were evaluated: gall index (GI), egg mass index (EMI), reproduction factor (RF), reproduction index (RI), and reduction of reproduction factor (RRF).

After removal from the experimental units, the roots were washed under running water and the gall was quantified using a Hansoros magnifying glass. They were then immersed in acid fuchsin solution for 10 minutes for better visualization of egg masses (Silva et al., 1988). The egg mass index (EMI) was obtained according to the methodology proposed by Taylor & Sasser (1978), where it employs a scale of scores from 0 to 5, which considers the number of galls or egg mass number, i.e., 0 = 0, 1-2 = 1, 3-10 = 2, 11-30 = 3, 31-100 = 4, and greater than 100 = 5.

The reproduction factor (RF) was obtained by the quotient between the final and initial nematode population (RF = Pfs + Pfr/Pi) as recommended by Oostenbrink (1966). While the reduction of reproduction factor (RRF) was obtained by the following formula: RRF = Frp - Frt / Frp × 100, where: Frp = reproduction factor in the species used as susceptibility standard; Frt = reproduction factor in the evaluated treatment (Moura & Régis, 1987).

The reproduction index (RI) of *M. javanica* was determined using nematode reproduction on tomato as the Standard witness (100%) compared to the cowpea treatments, according to the methodology established by Taylor (1967).

Statistical analysis

Data were subjected to multivariate normality analysis by Doornik & Hansen (2008) test ($p < 0.05$) and multivariate analysis of variance (MANOVA) by Wilks Lambda, Pillai Trace, Hotelling-Lawley Trace, and Roy Maximum Root at 5% significance levels. Then, the grouping of the treatments was performed using Ward's method (formation of homogeneous groups by the smallest internal variance), using the Euclidean distance and Pearson coefficient as reference. To discriminate the treatment groups as a function of the cowpea agronomic variables and *M. javanica* parasitism variables, canonical discriminant analysis was performed, which was represented in a biplot plot constructed for the first two canonical variables. Ninety five percent confidence ellipses were constructed in order to detect statistical differences ($p < 0.05$) between treatment groups. All analyses were performed with R software version 3.6.1 (R Core Team, 2021). Canonical discriminant analysis was performed using the candisc package (Friendly & Fox, 2015).

Results

There was significant difference ($p < 0.01$) between vectors of treatment means by the multivariate tests of Wilks Lambda, Pillai Trace, Hotelling-Lawley Trace, and Roy Maximum Root (Table 2).

The cluster analysis for the evaluated treatments allowed the division into four distinct groups, being: group I (F2-60 and

Table 2. Summary of the multivariate analysis of variance for the treatment average vectors.

Statistics	Value	[1]num Df	[2]den Df	Approx. F	Pr(>F)
Wilks Lambda	0.002246	80	122.73	2.4788	2.838e-06 **
Pillai Trace	3.4993	80	200	1.9438	0.0001005 **
Hotelling-Lawley Trace	13.577	80	130	2.7579	1.297e-07 **
Roy Maximum Root	5.7569	10	25	14.392	4.918e-08 **

[1] num Df: degrees of freedom of the numerator; [2] den Df: degrees of freedom of the denominator.

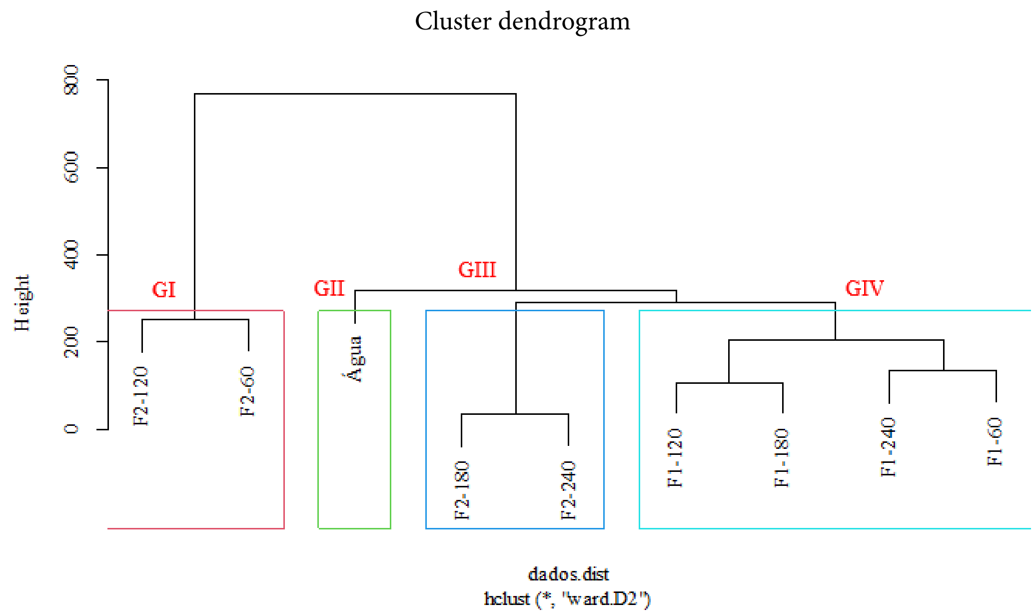

Figure 1. Grouping of the treatments in a dendrogram with the Euclidean distance considering the agronomic variables of cowpea plants and *M. javanica* parasitism variables.

F2-120), group II (Water), group III (F2-180 and F2-240), and group IV (F1-60, F1-120, F1-180, and F1-240) (Figure 1).

By canonical discriminant analysis, it was observed that the first two canonical variables explained 83.29% of the total variance contained in the nine original variables (Table 3). Analyzing the distribution of the weights of each variable, it is observed that the first canonical variable (Can.1) is most strongly correlated with the agronomic variables RV, RL, and FRM, and with the parasitism variables: NG, EM, ES, and JS, explaining 48.29% of the original variance. While the second canonical variable (Can.2) is more strongly correlated with the parasitism variables: ER, JR, and NGR, retaining 34.99% of the original variation.

Table 3. Coefficients, eigenvalues and proportion of variance explained by the canonical variables for the agronomic variables of cowpea plants and *M. javanica* parasitism variables.

Variables	Canonical variables	
	Can.1	Can.2
RV	**-0.45**	-0.24
RL	**-0.21**	-0.11
RFM	**-0.54**	-0.31
NG	**-0.85**	-0.21
EM	**-0.80**	-0.27
ES	**0.53**	-0.46
JS	**0.51**	0.04
ER	-0.16	**-0.48**
JR	-0.13	**-0.57**
NGR	0.04	**-0.48**
Eigenvalue	3.58	2.59
Explained variance (%)	48.29	34.99
Cumulative variance (%)	48.29	83.29

Agronomic variables: root volume (RV), root length (RL), and root fresh mass (RFM). *M. javanica* parasitism variables: number of gales (NG), egg mass (EM), eggs in the soil (ES), juveniles in the soil (JS), eggs on the root (ER), juveniles at root (JR), and nematoides per gram of root (NGR).

From the two-dimensional plane formed by the canonical variables Can1 and Can2 (Figure 2), it is observed that group II (composed only of the control treatment with added water) showed less root development of cowpea plants in relation to the other treatments, as evidenced by the lower means of the variables RV, RL, and RFM. In addition, the highest level of *M. javanica* parasitism was observed in the plants of the control treatment, by the highest averages of the variables ES, JS, ER, JR, and NGR.

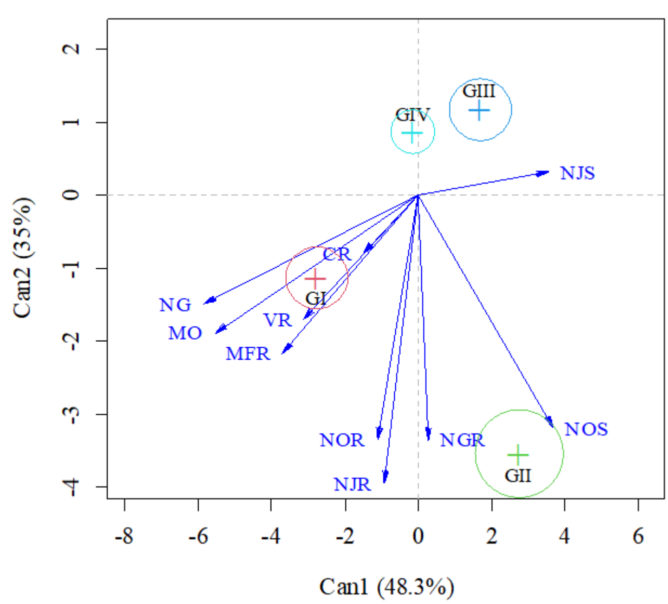

Figure 2. Graphical representation of the canonical discriminant analysis (canonical variables: Can1 and Can2) of the agronomic variables of cowpea bean: root volume (RV), root length (RL), and root fresh mass (RFM). *M. javanica* parasitism variables: number of gales (NG), egg mass (EM), eggs in the soil (ES), juveniles in the soil (JS), eggs on the root (ER), juveniles at root (JR), and nematoides per gram of root (NGR).

Among the treatments with the application of silicon doses and sources evaluated, group I treatments (F2-60 and F2-120) showed the highest mean root growth (RV, RL, and RFM) of cowpea, and provided the greatest reductions in the JS and ES variables of *M. javanica* parasitism (Figure 2). Despite its positive efficiency in root development and control of *M. javanica*, it still had a high number of galls and egg mass when compared to the other treatments and the control. While treatment group IV (F1-60, F1-120, F1-180, and F1-240) showed intermediate results for cowpea root growth and provided the greatest reductions in the ER, JR, and NGR variables of *M. javanica* parasitism. Treatment group III (F2-180 and F2-240) despite providing the lowest average root development of cowpea, were efficient in controlling *M. javanica* with significant reduction in all root parasitism variables (NG, EM, ER, JR, and NGR).

The plants when treated with the doses of Quimifol Silício®, presented scores between 3 and 4.75 for the gall and egg mass index (Table 3). When treated with the two highest doses of sodium silicate (180 and 240 g L^{-1}), the scores were between 2 and 2.75 (Table 3), which represents a large reduction in the number of galls and egg mass, showing that, although it did not completely control the number of galls and egg mass, it was efficient in protecting the root system of the plants when compared to the treatment without silicon application.

As for reduction of reproduction factor (RRF), reproduction factor (RF), and reduction index (RI), all silicon treatments, regardless of the source, were within the same group, being, moderately resistant, resistant and highly resistant for the aforementioned variables, respectively (Table 4). Bean plants when treated with the zero dose of silicon (water only), for RRF and RF, were classified as highly susceptible and susceptible, respectively (Table 4). These results show that silicon doses, regardless of the source, negatively influenced the reproduction of *M. javanica* in the bean crop, compared to the control (no silicate) and the positive control (tomato).

Discussion

The application of silicon (Si) showed positive results for root length, volume, and dry mass, and also in the control of *M. javanica* parasitism variables in cowpea plants. According to Guerriero et al. (2016), the addition of Si influences the processes related to the cell wall of dicots.

The severity of parasitism is reduced in many plants after the application of Si because it helps to avoid pathogenic penetration, as it forms physical barriers such as, for example, the formation of a double layer under the cuticle, consequently increasing systemic resistance. acquired (Rajput et al., 2021). Si binds to pectins, polyphenols, and hemicellulose present in the cell wall and increases the mechanical strength of the plant cell wall (Guerriero et al., 2016; Tripathi et al., 2021).

Besides strengthening cell walls, Si produces silicate papillae, which are responsible for inhibiting the spread of pathogenic structures. Therefore, although not an essential element, it increases plant vigor and plays important protective roles by behaving as a signaling factor, exerting positive effects by redirecting primary plant metabolism (Guerriero et al., 2016).

According to Alonso et al. (2022), the positive effects of Si on the control of potato nematodes are not only due to the formation of physical barriers, but also to the chemical action of increasing ascorbic acid and phenolic compounds in plant tissues, thus improving plant physiology. Oliveira et al. (2019) observed that exogenous application of some phenolic compounds increase the mortality of second-stage juveniles and decrease the number of *M. incognita* galls on tomato plants.

Alonso et al. (2022) also reported that increased Si uptake reduces the number of galls and eggs and the reproduction factor of *M. incognita*, and improves the physiological aspects of lettuce plants.

Some differences observed between the sources of Si for some variables, such as gall index and egg mass, where group III (F2-180 and F2-240), which includes the highest doses of sodium silicate showed better results, it is probably due to the characteristics of the sources used, since Quimifol Silício® (F1) has 10% of Si and 8.3% of K, while sodium silicate (F2) contains 29.4% of Si and 13.5% of Na.

Although group III (F2-180 and F2-240) was most efficient in pathogen control, group I treatments (F2-60 and F2-120) had the highest root growth averages (RV, RL, and RFM).

Table 4. Averages of gall index (GI), egg mass index (EMI), reduction of reproduction factor (RRF), reproduction factor (RF), and reduction index (RI) in soil infested with M. javanica, treated with doses of two silicon sources.

Sources	Doses (g L^{-1})	Parasitism variables				
		GI[1]	EMI[1]	RRF (%)[2]	RF[3]	RI[4] (%)
F1	60	4.73	4.75	93.02 (MR)	0.08 (R)	0.07 (HR)
F1	120	4.75	4.25	95.30 (MR)	0.05 (R)	0.05 (HR)
F1	180	3.50	3.00	94.86 (MR)	0.06 (R)	0.05 (HR)
F1	240	3.25	3.50	91.08 (MR)	0.10 (R)	0.09 (HR)
F2	60	5.10	5.00	90.64 (MR)	0.11 (R)	0.09 (HR)
F2	120	3.75	3.25	90.23 (MR)	0.12 (R)	0.10 (HR)
F2	180	2.00	2.00	95.31 (MR)	0.05 (R)	0.05 (HR)
F2	240	2.75	2.50	94.79 (MR)	0.06 (R)	0.05 (HR)
Water	0	4.25	4.10	15.34 (HS)	1.60 (S)	-
Tomato tree	0	4.50	4.20	0.00 (HS)	1.16 (S)	100.00 (S)

[1] Based on a proposal by Taylor & Sasser (1978), where: 0 = 0; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = greater than 100; [2] Based on the proposal by Moura & Régis (1987); [3] Based on the proposal by Oostenbrink (1966); [4] Based on the proposal by Taylor (1967). (R = Resistant; MR = Moderately resistant; HR = Highly resistant; S = Susceptible; HS = Highly susceptible).

According to Ribeiro et al. (2011), increasing doses of silicate can also present negative results on plants, such as inhibition of root growth. Thus, silicon application offers improvements, provided it is concentrated in adequate amounts in the cell wall, and also in the cytoplasm of the cells (Heine et al., 2005), which confers greater resistance to biotic and abiotic stresses.

In this sense, the accumulation of silicate in the roots can reduce injuries, favoring a better use of nutrients, water, and photo-assimilates in their full vegetative development, making them more vigorous than plants with pathogenic injuries. Results like this, reinforce the statements of Jackson & Taylor (1996), that the plant defense system consists of pre-existing physical and chemical barriers, as well as inductive defense responses, which are activated after infection by pathogens.

Conclusion

The application of Si is efficient in controlling *M. javanica* in cowpea plants, also favoring the development of the plants root system.

Compliance with Ethical Standards

Author contributions: Conceptualization: FAA; Data curation: EGS; Formal analysis: EGS, WLF, MLTL; Investigation: EGS, AMO; Methodology: FAA; Project administration: FAA; Resources: FAA; Supervision: MLTL; Validation: WLF, LMSX; Writing – original draft: EGS, AMO; Writing – review & editing: FAA; WLF, AMO, LMSX.

Conflict of interest: We declare for all intents and purposes that there is no conflict of interest.

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