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ORIGINAL ARTICLE

Silicon sources on *Cenostigma pyramidalis* seed quality[1]

Fontes de silício na qualidade de sementes de *Cenostigma pyramidalis*

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HIGHLIGHTS:

All silicon-based products control fungi of the genera Penicillium, Aspergillus *and* Cladosporium *in catingueira seeds.*
Rocksil® caused reductions in shoot length, root length, shoot dry mass, and root dry mass in the germination test.
Sifol® decreases the occurrence of fungi and does not interfere with physiological quality in seeds.

ABSTRACT: Seed quality is an essential characteristic for species reproduction. In this scenario, *Cenostigma pyramidalis* has important characteristics for the purpose of recovering degraded areas. However, because it is found in the Caatinga, this species tends to be more prone to infection by phytopathogens. Therefore, it is important to treat its seeds to prevent the incidence of fungi before and after planting. One of these alternatives is the use of silicon, which can contribute to increased vigor and disease control. In this scenario, the objective was to evaluate different sources of silicon in the control of naturally occurring fungi associated with *C. pyramidalis* seeds, and their physiological quality. The experiment was carried out at the Phytopathology Laboratory of the Federal University of Paraíba, Campus II, Areia, PB, Brazil. The seeds, after scarification to overcome dormancy, were treated with: T1 - Control; T2 - Captana, T3 - Agrosilício plus®; T4 - Rocksil®; T5 - Sifol®; T6 - Chelal®; and T7 - Bugram®. The experiment was conducted in a completely randomized design. The seeds were subjected to sanitary, germination, and emergence tests. 100 seeds were used per treatment for the germination and emergence tests in four replicates each with 25 seeds, while for the health test 10 replicates of 10 seeds each were used. All silicon sources were efficient in controlling *Aspergillus* spp., *Cladosporium* sp., and *Penicillium* sp. fungi in *C. pyramidalis* seeds. The treatment with Sifol® is recommended to control the incidence of fungi without affecting the physiological quality of seeds.

Key words: Catingueira, alternative control, seed pathology

RESUMO: A qualidade das sementes é uma característica essencial para a reprodução das espécies. *Cenostigma pyramidalis* apresenta características importantes para fins de recuperação de áreas degradadas. No entretanto, por ser encontrada na Caatinga, esta espécie tende a ser mais propensa à infecção por fitopatógenos. Portanto, é importante tratar tais sementes para evitar a incidência dos fungos antes e após o plantio. Uma dessas alternativa é o uso do silício que pode contribuir com o aumento do vigor e no controle de doenças. Neste cenário, objetivou-se avaliar diferentes fontes de silício no controle de fungos de ocorrência natural associados às sementes de *C. pyramidalis*, e sua qualidade fisiológica. O experimento foi desenvolvido no Laboratório de Fitopatologia, da Universidade Federal da Paraíba, Campus II, Areia-PB. As sementes, após escarificação para superação de dormência foram tratadas com: T1 - Controle; T2 - Captana, T3 - Agrosilício plus®; T4 - Rocksil®; T5 - Sifol®; T6 - Chelal®; T7 - Bugram®. O experimento foi conduzido em delineamento inteiramente casualizado. As sementes foram submetidas aos testes de sanidade, germinação e emergência. Foram utilizadas 100 sementes por tratamento para os testes de germinação e emergência em quatro repetições cada um com 25 sementes, enquanto para o teste de sanidade foram utilizadas 10 repetições de 10 sementes cada. Todas as fontes de silício foram eficientes no controle dos fungos *Aspergillus* spp., *Cladosporium* sp. e *Penicillium* sp. em sementes de *C. pyramidalis*. O tratamento com Sifol® é recomendado para controlar a incidência de fungos sem afetar a qualidade fisiológica das sementes.

Palavras-chave: Catingueira, controle alternativo, patologia de sementes

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Introduction

Cenostigma pyramidalis [Tul.] Gagnon & G. P. Lewis, commonly known in Brazil as Catingueira, belonging to the family Fabaceae, is a plant species endemic to the Caatinga geographic domain, with significant economic and environmental importance, showing several important features for the recovery of degraded areas, in addition to medicinal properties and possibility of use as forage (Freitas et al., 2019).

Tropical forests contain much more insects and microorganisms than plant species, living in a dynamic balance with plants and assuming the role of pests when these ecosystems are imbalanced (Remadevi, 2022). Therefore, ecosystem imbalances affect the dissemination and maintenance of plant species, especially when performed by seeds, favoring fungi associated with seeds more than those associated with other plant propagules (Saldanha et al., 2020).

Phytopathogenic microorganisms cause tissue damage, potentially leading to seed rot and death, decreasing germination and causing seedling anomalies and damage (Nóbrega & Nascimento, 2020). Therefore, the analysis of sanitary seed quality is extremely important to avoid the dissemination of phytopathogens into new areas free of disease incidence, and seed treatment can eradicate or reduce future damage caused by these microorganisms during seedling production (Gomes et al., 2019)

In this scenario, in order to achieve good seedling development, it is essential to perform pre-treatments to prevent the incidence of fungi that compromise plant development. Therefore, fungicides have been a widespread recommendation, even though agrochemicals can affect the beneficial microbial population present in the ecosystem, besides causing damage to the environment, humans, and animals (Mahamod & Ismael, 2020).

An alternative with less environmental impact to production systems that also reduces the use of agrochemicals is the application of silicon to crops (Machado & Queiroz, 2018). Dhakate et al. (2022) stressed that the use of silicon via seeds can contribute to increasing crop yield. Silicon application has also increased seed quality, reduced the incidence of fungal diseases, and favored soil fertility and plant development (Vieira et al., 2022). Nunes et al. (2023) found silicon to be efficient in reducing the severity of anthracnose (*Colletotrichum lindemuthianum*) when applied to the upper middle third of common bean (*Phaseolus vulgaris* L.) leaves with 60 and 65 days after emergence.

In this scenario, the present study aims to evaluate different sources of silicon in the control of naturally occurring fungi associated with *C. pyramidalis* seeds, and their physiological quality.

Material and Methods

The experiment was conducted at the Laboratory of Phytopathology (LAFIT) of the Center of Agricultural Sciences (CCA) of the Federal University of Paraíba (UFPB), Campus II – Areia (6° 57’ 48” S, 35° 41’ 30” W, and altitude of 618 m), State of Paraíba, Brazil. The study was carried out from October 2 to November 3, 2022.

Seeds of Catingueira (*C. pyramidalis*) were provided by the Seed Network of the São Francisco Integration Project (Rede de Sementes - PISF), in the city of Petrolina, PE. The seeds used were obtained from healthy trees free from pest and disease attack, located in Juazeiro, BA, on the collection date of July 13, 2022. The seeds were cut on the opposite side of the hilum to overcome physical dormancy. Subsequently, the seeds were disinfected with 1% sodium hypochlorite for three minutes.

The seeds were treated with the following treatments: T1 - control (sterile distilled water – SDW); T2 – Captana® (240 g 100kg^{-1}), T3 - Agrosilício plus® (3 g L^{-1})(Si = 10.5%, Ca = 25%, and Mg = 6%); T4 - Rocksil® (3 g L^{-1}) (Si = 17 %, Ca = 12%, and K = 3%); T5 - 12% potassium silicate, Sifol® (10 mL L^{-1})(Si = 12% and K_2O= 15%); T6 - 5.9% silicate, Chelal® Si (3.3 mL L^{-1}) (K_2O = 5.9%, Si = 5.9 %); T7 - Bugram® (1 g 100kg^{-1}) (SiO_2 = 94.6%, Al_2O_3 = 3.38%, TiO_2 = 0.21%, Fe_2O = 0.23%, CaO = 0.42%, MgO = 0.44%, Na_2O = 0.18%, K_2O = 0.11%, MnO = 0.01 %, P_2O_6 = 0.01%). The treatment with Captana® was calculated based on seed weight, and the other treatments were diluted in 100 mL of SDW.

The sanitation test was conducted by soaking the seeds for 5 minutes in solutions with the concentrations of the respective treatments. The fungicide Captana® was applied by direct contact with the seed epidermis. The treated seeds were laid in Petri dishes (9 cm diameter) containing a double layer of sterile filter paper moistened with SDW, and stored in the incubation room (25 ± 2 °C, 12 hours photoperiod) for seven days. After this period, the identification of the fungi present in the seeds was performed with a light microscope by comparing the morphological structures with the descriptions available in the specialized literature (Seifert et al., 2011). The results were expressed as percentage of infected seeds.

Physiological quality was determined using the same treatments mentioned before. The seeds were distributed onto two Germitest® paper sheets covered with a third sheet and organized in rolls, which were moistened with an SDW volume corresponding to 2.5 times the dry paper weight. The rolls were then put into transparent polystyrene bags in order to avoid water loss by evaporation and put in BOD (*Biochemical Oxygen Demand*) incubators at 25 ± 2 °C with a 12 hours photoperiod. Germinated seeds were evaluated after 14 days, and the numbers of normal seeds, hard seeds, and dead seeds were counted.

The first count occurred simultaneously to the germination test, by counting all seeds germinated on the seventh day after sowing (BRASIL, 2013). The percentage of germinated seeds was determined by the following equation:

$$PGS = \left(\frac{N_2}{N_1}\right) \times 100$$

where: PGS = percentage of germinated seeds, N_2 = number of germinated seeds, and N_1= number of seeds under test. The germination speed index (GSI) was calculated by daily recording the number of germinated seeds (Maguire, 1962).

The emergence test was conducted in the plant nursery of the Phytopathology Laboratory of CCA/UFPB. The seeds were sown in polystyrene trays containing sterile Mecplant®

commercial substrate and irrigated daily. The emerged seedlings were counted at 24-hour intervals. The first count was performed concomitantly with the count of the number of seedlings emerged on the seventh day after sowing (BRASIL, 2009), and the percentage of emerged seedlings (PE) was calculated. The emergence speed index (ESI) was calculated by daily recording the number of emerged seedlings until their stabilization, which occurred on the 14th day, and then applying the equation proposed by Maguire (1962).

At the end of the germination and emergence tests, shoot length (SL) and root length (RL) were measured with a ruler, and the results were expressed in centimeters. To determine shoot dry mass (SDM) and root dry mass (RDM), the respective parts were oven-dried in a forced air oven at 65 °C for 48 hours and subsequently weighed on an analytical scale (0.0001 g). The results were expressed in g per seedling.

The experiment was conducted in a completely randomized design, consisting of seven treatments. For the sanitation test, 10 seeds were distributed in 10 replicates, and for the germination and emergence tests, there were four replicates with 25 seeds. The data were subjected to analysis of variance (ANOVA) and comparison of means by Scott-Knott's test at 0.05 probability level. Pathogen incidence data were transformed into ($\sqrt{x} + 1$), as described by Bartlett (1947). The statistical procedures were processed with R software (R Core Team, 2024).

RESULTS AND DISCUSSION

According to the results of the analysis of variance (Table 1), there was a significant effect of the treatments on the incidence of *Penicillium* sp. (PEN), *Aspergillus* spp. (ASP), and *Cladosporium* sp. (CLA). For the germination test, significant differences were observed for the germination percentage (G), first germination count (FCG), dead seeds (DS), germination speed index (GSI), shoot length (SL), shoot dry mass (SDM), and root dry mass (RDM). For the emergence test, the treatments significantly influenced the emergence percentage (EP), emergence speed index (ESI), root length (RL), and shoot dry mass (SDM) (Table 1).

Table 1. Summary of analysis of variance for *Penicillium* sp. (PEN), *Aspergillus* spp. (ASP), *Fusarium* sp. (FUS), *Cladosporium* sp. (CLA), *Epicoccum* sp. (EPI) of germination percentage (GP), first germination count (FGC), dead seeds (DS), germination speed index (GSI), emergence (EM), emergence speed index (ESI), and shoot length (SL), root length (RL), shoot dry mass (SDM), and root dry mass (RDM) of germination and emergence tests

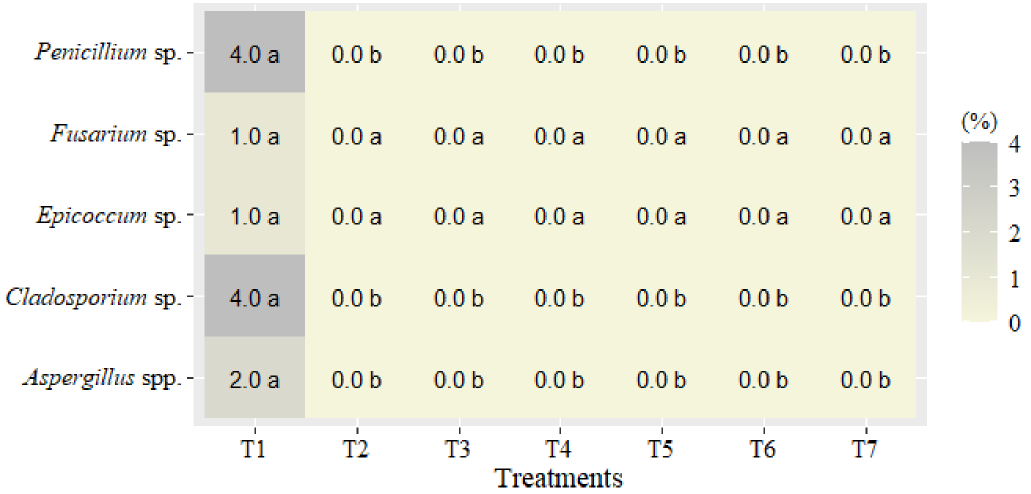
Sources of variation	DF	Mean squares				
		PEN	ASP	FUS	CLA	EPI
Treatments	6	0.0392**	0.0098*	0.0025 ns	0.0348**	0.0025 ns
Residual	63	0.0065	0.0044	0.0025	0.0101	0.0025
CV (%)		7.90	6.52	4.92	9.82	4.92
		GP	FGC	DS	GSI	SL-G
Treatments	6	525.14**	329.14**	525.14**	7.45**	19.39**
Residual	21	126.48	64.95	120.76	1.59	1.23
CV (%)		28.52	43.73	18.14	33.94	16.76
		RL-G	SDM-G	RDM-G	EM	ESI
Treatments	6	4.11ns	0.0017**	0.0003**	314.67**	0.62**
Residual	21	1.65	0.0003	0.0001	48.19	0.12
CV (%)		29.10	35.76	34.34	27.77	31.80
		SL-E	RL-E	SDM-E	RDM-E	
Treatments	6	3.98 ns	2.45*	0.0520**	0.0012 ns	
Residual	21	2.62	0.86	0.0114	0.0005	
CV (%)		16.80	8.85	49.01	44.91	

ns, * and ** - Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively; CV - Coefficient of variation; DF - Degrees of freedom; SL, RL, SDM and RDM - Germination (-G) and emergence (-E) tests, respectively

In the evaluation of sanitary quality, five fungal genera associated with Catingueira seeds were identified (Figure 1). The incidence of the fungal genera *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium*, and *Epicoccum* was observed.

Regarding the incidence of *Aspergillus* spp., it was observed that silicon-based treatments provided an antifungal effect when compared to the control, not differing from the fungicide (Figure 1).

Similar to the observations with Catingueira seeds, other forest species such as *Bauhinia variegata* Linn. (Gomes et al., 2019) have also shown the presence of the fungal genera *Aspergillus* and *Penicillium*. When associated with seeds during storage, these fungi cause deterioration and thus reduce seed viability, in addition to produce toxic mycotoxins (Pfliegler al., 2020).



Means followed by the same letters do not differ by Scott-Knott's test ($p \leq 0.05$). T1 - Control (sterile distilled water – SDW); T2 - Captana® (240 g 100kg^{-1}), T3 - Agrosilício plus® (3 g L^{-1}); T4 - Rocksil® (3 g L^{-1}); T5 - 12% potassium silicate, Sifol® (10 mL L^{-1}); T6 - 5.9% silicate, Chelal® Si (3.3 mL L^{-1}); T7 - Bugram® (1 g 100kg^{-1})

Figure 1. Incidence of fungal genera in Catingueira (*Cenostigma pyramidalis*) seeds treated with silicon sources

As to the incidence of *Fusarium* sp. and *Epicoccum* sp., no statistical difference was observed between treatments (Figure 1). The occurrence of the *Fusarium* genus, even at low incidence, can cause infections in seeds and seedlings of forest species.

As a soil pathogen, this fungus causes root and stem rot and, when present in seeds during storage, this microorganism produces a mycotoxin that leads to the total loss of germinative capacity (Farias et al., 2023).

When evaluating the control of fungi in rice (*Oryza sativa*) seeds, using silicon sources, Tunes et al. (2014) observed a 70% reduction in the incidence of *Epicoccum* sp., with the highest dose, which differs from the incidence value found in this study.

With regard to the genera *Penicillium* and *Cladosporium*, all treatments had a significant difference when compared to the control, showing efficacy (Figure 1). The results showed that silicon-based treatments may have contributed to the inhibition of fungal growth, which resulted in effective control of these pathogens. This effect may be associated with the antifungal action of silicon, which, when interacting with the plasma membrane of these microorganisms, can cause damage to its structure (Coskun et al., 2019).

One of the positive points of using silicon is the higher disease resistance achieved as this element acts in biochemical, physiological, and photosynthetic plant processes (Coskun et al., 2019).

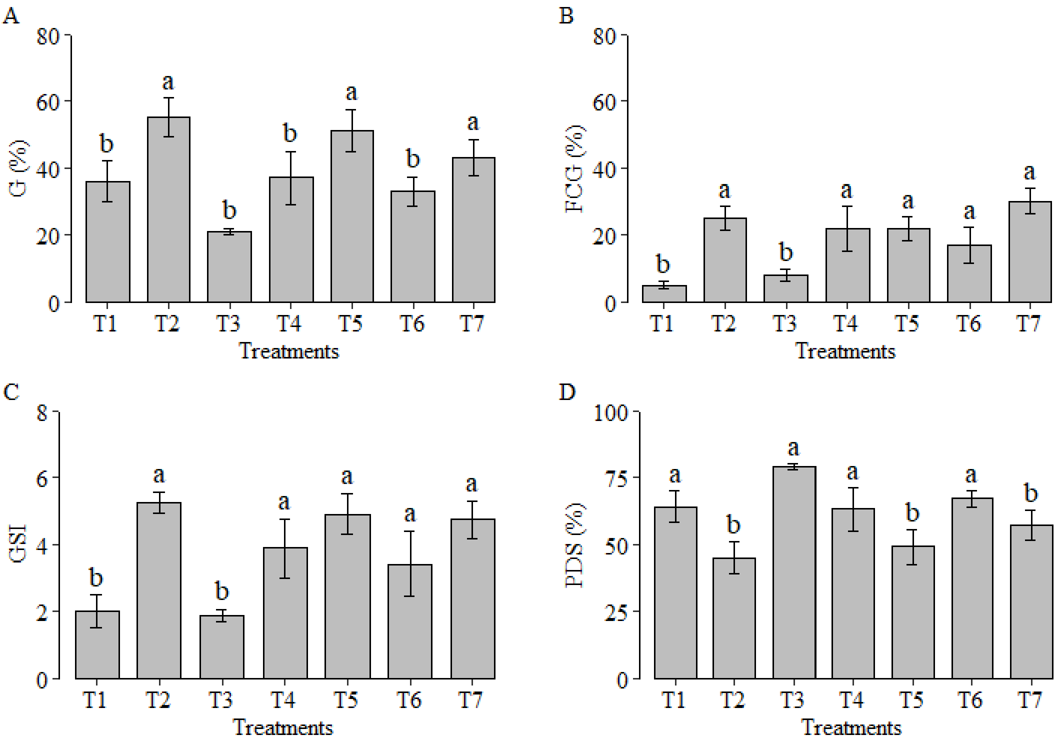
The physiological conditioning with 12% potassium silicate Sifol® and Bugram® improved the germination performance of *C. pyramidalis* seeds (Figure 2A). These treatments showed similar results to the application of fungicide Captana®, increasing germination, differing statistically from the other treatments.

Silicon can be derived from steel remains, from the reaction of limestone with silica, and as a by-product of burnt rice husk. Several Si-rich sources are cheaper due to their availability in industrial waste (Migliorini et al., 2021). However, the way these sources are made available and the amount with which they are supplied are different. Therefore, certain Si concentrations may have beneficial effects, such as reduced production of reactive oxygen species and significant increments in seed germination and antioxidant enzyme activity (Naidu et al., 2023).

In the first germination count (FCG), the seeds treated with Bugram® showed higher radicle emergence speeds, as well as those under the treatments with Captana®, Rocksil®, Sifol®, and Chelal®, with values ranging from 22 to 30%. The control treatment and the one with Agrosilício plus® manifested a similar behavior, with lower FCG percentages (Figure 2B).

With regard to the germination speed index (GSI), the treatment with Captana®, followed by the treatments with Rocksil®, Sifol®, Chelal®, and Bugram®, yielded the best results (Figure 2C). For this variable, Agrosilício plus® showed an inferior mean value compared to the other treatments, but performed similar to the control.

When analyzing the main functions of Si in plants, Gama et al. (2021) found that this element promotes growth and increased yield, demonstrating also its plant protection action. In another study, using silicon in soybean (*Glycine max* L.) seeds, the authors found that quality was not affected and observed an increase in seed vigor (Machado & Queiroz, 2018).



Means followed by the same letters do not differ by Scott-Knott's test ($p \leq 0.05$). T1 - Control (sterile distilled water – SDW); T2 - Captana® (240 g 100kg^{-1}), T3 - Agrosilício plus® (3 g L^{-1}); T4 - Rocksil® (3 g L^{-1}); T5 - 12% potassium silicate, Sifol® (10 mL L^{-1}); T6 - 5.9% silicate, Chelal® Si (3.3 mL L^{-1}); T7 - Bugram® (1 g 100kg^{-1}). G - Germination percentage, FCG - First count of germinated seeds, GSI - Germination speed index, and PDS - Percentage of dead seeds

Figure 2. Evaluation of the physiological quality of Catingueira (*Cenostigma pyramidalis*) seedlings treated with commercial silicon sources. A. Germination percentage (G). B. First count of germinated seeds (FCG). C. Germination speed index (GSI). D. Percentage of dead seeds (PDS)

The percentage of dead seeds was influenced by the incidence of fungi during the germination test, which caused seed mortality (Figure 2D). The treatments with the highest percentage of dead seeds were the ones with Agrosilício plus®, Rocksil®, and Chelal®, with 60 to 80% seed mortality, not differing statistically from the control. The treatments with Captana® and Sifol® had lower death rates, with means under 50%.

Although silicon application creates a physical barrier to pre-infection by pathogens through the formation of a double layer of amorphous silica and the salinization of cells, these characteristics prevent infection (Santos et al., 2021) but are not sufficient to prevent the incidence of *Penicillium* sp., and *Aspergillus* spp., which are possibly the cause for the high mortality of these seeds as they can interfere with germination, emergence of seedlings, and their development in the field (Rosário et al., 2022).

For shoot length, Bugram®, Captana®, and Agrosilício plus® achieved the highest results, whereas Rocksil® showed the lowest value (Figure 3A). The treatments with Rocksil® and Chelal® influenced the reduction of root length, showing the lowest values (Figure 3B). Results divergent from those obtained in the present study were found by Gou et al. (2020) when analyzing the influence of silicon on the initial development of soybean (*Cucumis sativus* L).

For shoot dry mass, the control treatment and the treatments with Agrosilício plus®, Sifol®, Chelal®, and Bugram® differed from fungicide and Rocksil®, promoting an increase in this parameter. On the other hand, the fungicide and Rocksil® reduced seedling development (Figure 3C). For root dry mass, Agrosilício plus®, Rocksil®, and Chelal® showed the lowest results, interfering with the full seedling development (Figure 3D).

In general, plants with more roots are less likely to suffer stress caused by abiotic or biotic factors (Teshome et al., 2020). This fact may be directly related to the results found in this study since several genera of phytopathogenic fungi were associated with the seeds, which may have compromised root development.

It is possible to state that silicon, even though it is not considered an essential element, provides several benefits, whether in tolerance to biotic stresses, or in the ability to promote an increase in the physiological development of seedlings (Santos et al., 2021).

The treatments Captana®, Rocksil®, and Sifol® showed higher values than Agrosilício plus® (Figure 4A). With regard to the emergence speed index, treatments Captana® and Sifol® showed higher speeds compared to Agrosilício plus® (Figure 4B). Therefore, both the fungicide and the commercial sources of silicon, Rocksil® and Sifol®, acted on the fungi present in the seeds in a way that promoted greater emergence percentages and speeds of *C. pyramidalis*.

Machado & Queiroz (2018) analyzed soybean seeds treated with silicon and observed that the seeds that received the highest dose of silicate showed greater vigor. Similar results were found by Gou et al. (2020), in whose study rice seeds treated with silicon showed greater vigor. According to Santos et al. (2021), Si provides numerous benefits, e.g., greater tolerance to biotic and abiotic stresses, directly acting on plant physiology and participating in important chemical processes in the soil.

The variable shoot length was not influenced by the treatments applied via seed (Figure 5A). However, significant

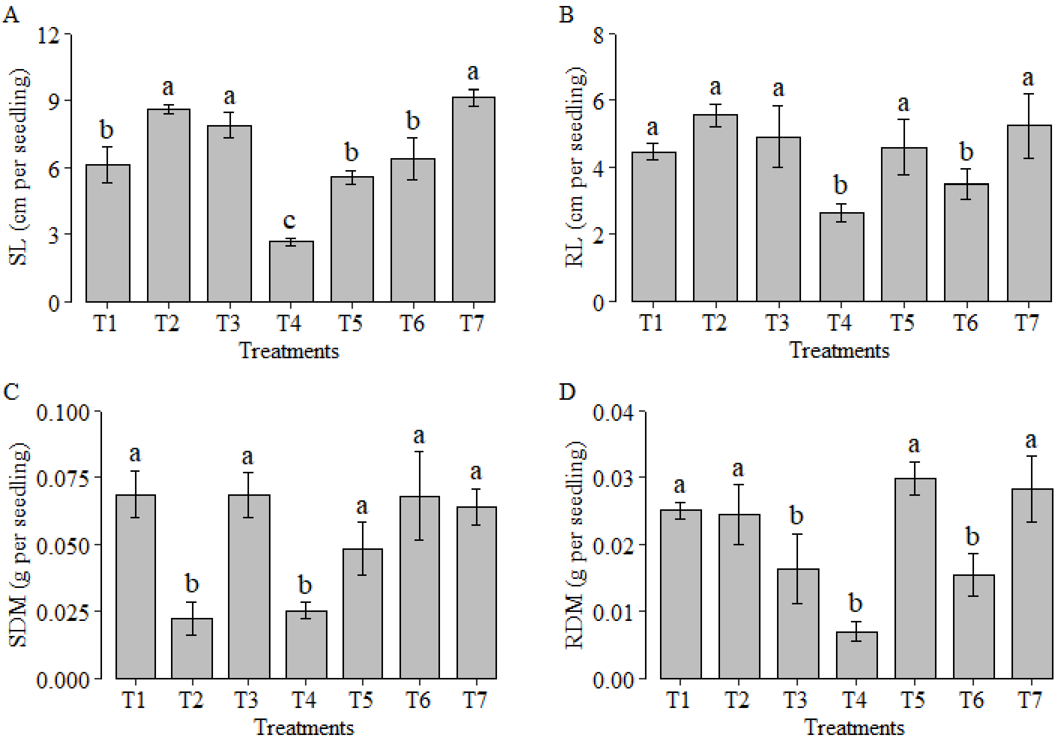


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Figure 3. Physiological quality of Catingueira (*Cenostigma pyramidalis*) seedlings grown from seeds treated with commercial silicon sources. A. Shoot length (SL), B. Root length (RL), C. Shoot dry mass (SDM), and D. Root dry mass (RDM)

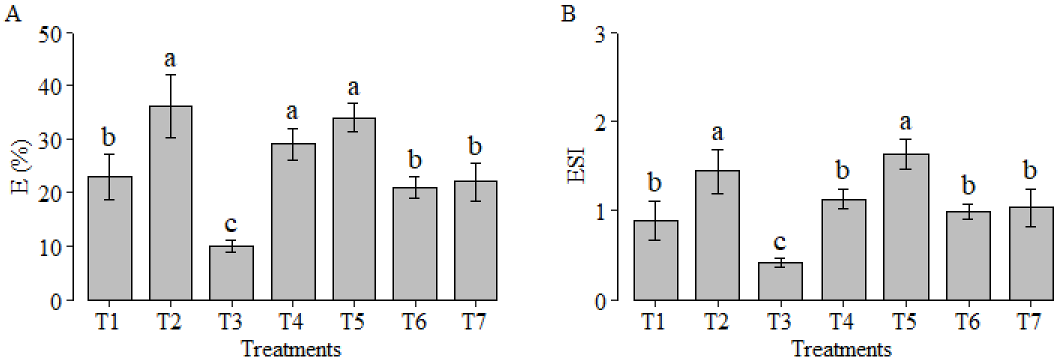

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Figure 4. Evaluation of the physiological quality of Catingueira (*Cenostigma pyramidalis*) seeds treated with commercial silicon sources. A. Mean emergence (E%). B. Emergence speed index (ESI)

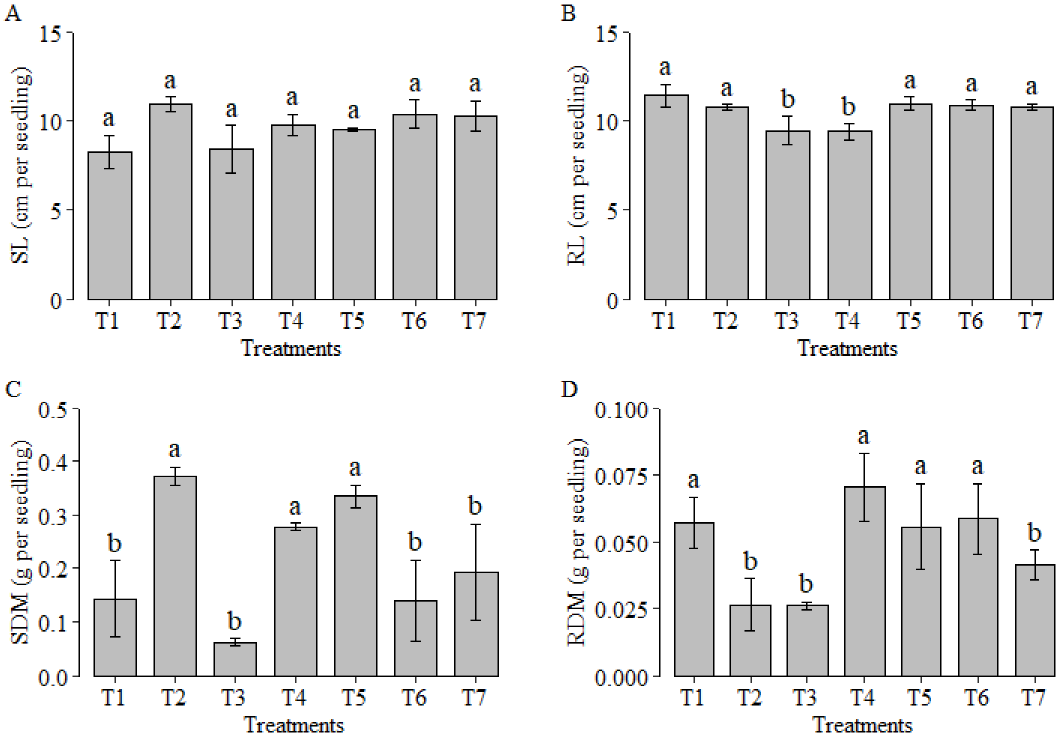

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Figure 5. Emergence test of Catingueira (*Cenostigma pyramidalis*) seeds treated with commercial silicon sources. A. Shoot length (SL), B. Root length (RL), C. Shoot dry mass. D. Root dry mass

differences were found between the treatments with regard to root length (Figure 5B). Lower root length values were obtained with the Agrosilício plus® and Rocksil®, differing statistically from the other treatments.

Corroborating these results, treatments applied to soybean (*Glycine max*) seeds, with different doses of silicon, did not cause significant differences in these variables (Silva, 2021). These data differ from those obtained by Sun et al. (2021), who observed that treatments with silicon doses promoted greater root development. However, other factors such as the genetic potential of the cultivars and environmental conditions must also be considered.

When evaluating shoot dry mass, treatments Captana®, Rocksil®, and Sifol® had higher values when compared to the other treatments (Figure 5C). Regarding root dry mass, it was observed that the treatments Rocksil®, Sifol®, and Chelal® had the best results compared to the other treatments and did not differ significantly from the control treatment (Figure 5D).

Although studies still do not explain exactly how silicon acts on molecular mechanisms and metabolic pathways in seeds, it is possible to observe that this element promotes benefits in germination, especially when lettuce seeds are subjected to stress conditions (Pereira et al., 2021).

CONCLUSIONS

1. All silicon sources were efficient in controlling *Aspergillus* spp., *Cladosporium* sp., and *Penicillium* sp. fungi in *Cenostigma pyramidalis* seeds.

2. The treatment with Sifol® is recommended to control the incidence of fungi without affecting the physiological quality of seeds.

Contribution of authors: Lucy Gleide da Silva: conception, data collection, manuscript preparation, discussion of results. Juciely Gomes da Silva: conception, data collection, manuscript preparation, discussion of results. Rayane Ellen de Oliveira Jeronimo: conception, data collection, manuscript preparation, discussion of results. Ana Karoliny de Assis Medeiros: conception, data collection. Hilderlande Florêncio da Silva: data analysis. Riselane de Lucena Alcântara Bruno: conception, review. Luciana Cordeiro do Nascimento: conception, review.

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