



EFFECT OF SODIUM CHLORIDE ON GERMINATION AND VIGOR OF *Cajanus cajan* (L.) MILLSPAUGH SEEDS

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Keywords:

Saline stress
Fabaceae
Guandu
Osmotic Potential

ABSTRACT

Cajanus cajan is used as fertilizer or as food for human and animal. Since salinization of soils is a worldwide problem, it is necessary to look for resistant species. Thus, this study aimed to evaluate the effect of NaCl on germination, emergence and vigor of *C. cajan*. Therefore, seeds of the pintadinho cultivar and five concentrations of NaCl (1.5; 3.0; 4.5; 6.0; and 7.5 dSm⁻¹) and control were used. A completely randomized design was performed with four repetitions of 50 seeds for each treatment. Data were submitted to analysis of variance, using the F test to compare mean squares, and the polynomial regression analysis for quantitative effects. The progressive addition of salt had no significant effect on germination and emergence of *C. cajan* seedlings, however it reduced its vigor.

Palavras-chave:

Estresse salino
Fabaceae
Guandu
Potencial osmótico

EFEITO DO CLORETO DE SÓDIO NA GERMINAÇÃO E VIGOR DE SEMENTES DE *Cajanus cajan* (L.) MILLSPAUGH

RESUMO

Cajanus cajan é utilizada como adubo, alimentação humana e animal. Sabido que a salinização dos solos é um problema mundial necessita-se pesquisar espécies resistentes. Assim o objetivo foi avaliar o efeito do NaCl na germinação, emergência e vigor de *C. cajan*. Para isso foram utilizadas sementes da cultivar pintadinho e cinco concentrações de NaCl (1,5; 3,0; 4,5; 6,0; e 7,5 dSm⁻¹) e controle. O delineamento experimental utilizado foi inteiramente ao acaso, com quatro repetições de 50 sementes para cada tratamento. Os dados foram submetidos à análise de variância, utilizando-se o teste F para comparação dos quadrados médios, sendo que para os efeitos quantitativos foi realizada análise de regressão polinomial. A adição progressiva do sal não causa efeito significativo à germinação e emergência de plântulas de *C. cajan*, entretanto reduz o vigor.

INTRODUCTION

Cajanus cajan (L.) Millspaugh is a shrubby species popularly known in Brazil as guandu bean. It has the potential to be used in green fertilization and in the suppression of spontaneous plants. This species has also the ability to promote physical protection to the soil, due to the rapid biomass production and acts in the chemical restoration of soils. As it is a Fabaceae, it plays an important role in biological nitrogen fixation. In addition, this species can be used for human and animal food (RAYOL & ALVINO-RAYOL, 2012; AZEVEDO *et al.*, 2007).

Salinity is evident in the Northeast region, being enhanced by the high evaporation rates of water present in the soil and also by the low rainfall, which contributes to the accumulation of salts on the soil surface (LIMA JUNIOR & SILVA, 2010). Another aggravating factor is the irrigated agriculture, which, when used multiple times in degraded areas, with saline water, compromises soil quality and causes economic losses (RIBEIRO, 2010).

Soil salinity is a worldwide problem and it is considered a barrier to food production, since it affects the physiological processes of plants and compromises their development and reproduction. Therefore, researches must be performed to verify the tolerance of several species in order to select them for cultivation in these soils.

In common bean (*Phaseolus vulgaris* L.) and cowpea bean (*Vigna unguiculata* L.) the effect of salinity causes a reduction in the length of the hypocotyl and the root system, causing an increase in diameter (DE PAULA *et al.*, 1994), a reduction of leaf area and dry mass (XAVIER *et al.*, 2014; BARBOSA *et al.*, 2012), beyond leaf wilting (NIEMAN, 1964). In a study performed by Pinheiro *et al.* (2013), using *C. cajan*, the final germination values for seeds that were not submitted to salt stress reached 88%. This value reduced to 48% at the osmotic potential of -1.5 MPa. This same behavior was observed for the first germination count, which presented a maximum value of 84% for the control treatment, reducing to 41% of germination at the concentration of -1.5 MPa.

Thus, the aim of this study was to evaluate the effect caused by NaCl on the germination, emergence and vigor of *Cajanus cajan* (L.) Millspaugh, Pintadinho cultivar, from family farming in Solânea, PB.

MATERIAL AND METHODS

The study was performed using *C. cajan* seeds of the “pintadinho” cultivar, originated from family farming in Solânea – PB. The seeds were harvested, processed and stored in PET bottles at room temperature for a maximum of 30 days until starting the experiment.

The water content was determined in four repetitions, with 20 seeds each, placed in aluminum capsules and in an oven at 105 ± 3 °C for 24 hours, and the results were expressed in percentage (BRASIL, 2009).

The saline solutions were composed of sodium chloride (NaCl) in different concentrations: 0.0 (control); 1.5; 3.0; 4.5; 6.0; and 7.5 dSm⁻¹, according to the methodology proposed by Richards (1980). The germination test was performed in four repetitions of 50 seeds each, disinfected with a 10% hypochlorite solution for three minutes and washed in distilled water. Then, the seeds were distributed on the Germitest® paper substrate, which was moistened, for each concentration, with saline solution (NaCl) in an amount equivalent to three times the weight (g) of the dry paper, without further addition of water. The rolls were packed in 0.04 mm thick transparent plastic bags, in order to avoid loss of water by evaporation.

The emergence test was performed in four repetitions of 50 seeds, disinfected with a 10% hypochlorite solution for three minutes and washed with distilled water. Then, they were distributed in gerbox-type boxes containing washed sand as substrate, which was autoclaved and dried in an oven. Thereafter, the seeds were moistened with volumes of saline solution, for each concentration. The solution was added up to 60% of the water retention capacity of the soil.

The germination and emergence tests were conducted in a Biological Oxygen Demand (B.O.D.) germinator set at 25 °C, 30 °C, using daylight fluorescent lamps (4 x 20 W), with a 12-

hour photoperiod. The counts were performed from the fourth to the tenth day after sowing, according to the criteria established by Brasil (2009).

The first germination counts (FGC) and emergence (FEC) counts were conducted together with the germination and emergence tests, respectively, computing the number of seeds germinated on the fourth day after the test started and the results were expressed as percentage.

The germination (GSI) and emergence (GSE) speed indices, were determined together with the germination and emergence tests, through daily counts, from the fourth to the tenth day after sowing. The formula proposed by Maguire (1962), $IVE = (G1/N1) + (G2/N2) + \dots + (Gn/Nn)$, was used to calculate the indices.

The evaluations were performed daily after the installation of the test, from the fourth to the tenth day, considering as germinated seeds those that emitted the primary and hypocotyl root and for emergence, the emission of the hypocotyl as well as those that were apparently healthy according to the criteria of Brasil (2009), whose results were expressed as percentage. The results were expressed in percentage.

Seedlings that were damaged, without the presence of main root or aerial part or with any other deformed structure were considered as abnormal seedlings, according to the criteria established by Brasil (2009).

The length, diameter and dry mass of seedlings were obtained at the end of the germination test on the tenth day after sowing. In the normal seedlings of each repetition, the root, the aerial part and the lap thickness (diameter) were measured using a digital caliper. The results of the root and aerial part were expressed in centimeters and the lap thickness in millimeters.

The aerial parts and roots of the normal seedlings were used to determine the dry mass. They were placed separately by repetition (without the cotyledons) in Kraft paper bags and placed to the forced ventilation oven under regulated temperature at 65°C until approximately 72 hours. After this period, the samples were weighed on an analytical balance with a precision of 0.001g, and the results were expressed in grams.

The Dickson Quality Index (DQI) was determined as a function of aerial part height (PH),

lap diameter (LD), dry phytomass of aerial part (DPAP) which is given by the sum of the dry mass of the stem (DMS) and the dry phytomass of the leaf (DPL) and the dry phytomass of the roots (DPR), through the equation provided by DICKSON *et al.*, (1960), $DQI = (g) / DMT(cm)(LT(mm) + LD(g)DPR(g)$

The completely randomized design was performed in four repetitions of 50 seeds for each treatment. The data were submitted to analysis of variance, using the F test to compare mean squares, and polynomial regression analysis for quantitative effects using the SISVAR software.

RESULTS AND DISCUSSION

The water content of *C. cajan* seeds was 11.25%, which, according to Alves and Lins (2003) is within the recommended standards for a good storage condition of orthodox seeds.

According to the analysis of variance data, no significant difference was observed among NaCl concentrations and the analyzed variables: germination (G%), germination speed index (GSI), normal seedlings (NS%), abnormal seedlings (AS%), and dead seeds (DS%). A significant influence was observed only among treatments with different concentrations of NaCl on the first count of germinated seeds (CGS) at 5% probability (CV% 8.12).

The non-significance in the germination test indicated that despite the harmful effect of salt on the vegetative structures, the seeds of *C. cajan* were able to reach high germination percentages even with the increase of salt concentrations. Reinforcing this assertion, Souza *et al.* (2022), in their research, concluded that salinity induced by NaCl did not negatively affect seed germination of *Cajanus cajan* cv. Khaki, indicating that the species show tolerance to saline stress at osmotic potentials from -0.2 to -0.8 MPa.

As can be seen in Figure 1, there was a reduction in the percentage of the first germination count at higher concentrations of NaCl, and the lowest percentage was observed at the concentration of 6.5 dSm⁻¹. Pinheiro *et al.* (2013), testing different concentrations of NaCl in the germination of *C. cajan* seeds, verified that for the variable first germination count, in the control treatment, the

percentage of germination obtained was 84%, reaching to 41% in the concentration of -1.5 MPa.

In Table 1 is shown that the length of the aerial part and primary root, diameter and dry weight of the aerial part were affected by the addition of salt to the substrate. However, the dry weight of the main root and the Dickson index were not impaired. This result shows that this species *C. cajan*, under the conditions of this experiment, presented a moderate capacity to develop, even in conditions of saline stress environments, caused by NaCl.

Among the NaCl concentrations studied in the germination test with *Cajanus cajan* seeds, it can be noted that for all analyzed variables, except for RDM and DQI, significant responses occurred with the addition of NaCl. In Figure 2, it is possible to observe that the highest value for the lengths of the aerial part and root of the *C. cajan* seedlings was obtained using saline solution concentration of 1.5 dSm⁻¹. From this value, the tendency was of reduction, which characterizes the deleterious effects of salt in higher concentrations.

Similar results were observed for the dry mass of the aerial part and lap diameter, which showed a reduction in development with the addition of salt. Monteiro et al. (2014) in a research with cultivars of *C. cajan* submitted to osmotic stress and exogenous putrescine, observed that the levels of proline in the aerial part and root increased with the reduction of water availability or with the increase in salinity. Furthermore, the roots of BRS Mandarin cultivar were especially sensitive to salinity, mainly from the saline concentration of 20 mmol L⁻¹.

According to Schafranski et al. (2019), salinity had a negative effect on the two bean cultivars, *Phaseolus vulgaris* L., (black and carioca) analyzed in their work, with an increase in NaCl concentrations, affecting the physiological quality of the seeds. There was a progressive decrease in the growth of bean seedlings with the increase of sodium chloride addition.

In Figure 2, it can also be observed that the increase in NaCl concentrations promoted a reduction in the dry mass of the aerial part, mainly from the 7.3 dSm⁻¹ concentration. According to

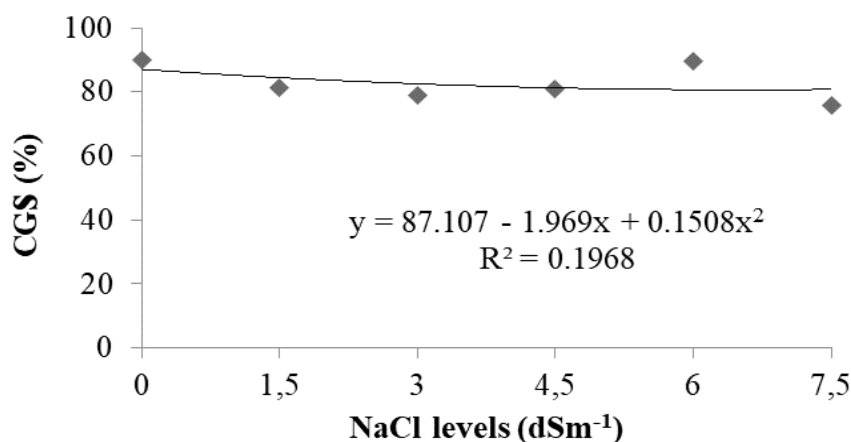


Figure 1. First germination count (FGC%) of *Cajanus cajan* seeds submitted to different NaCl concentrations.

Table 1. Summary of Analysis of variance for aerial part length (APL), root length (LR), lap diameter (LD), aerial part dry mass (APDM), root dry mass (RDM), Dickson Quality Index (DQI), from the germination test with *Cajanus cajan* seeds

SV	DF	F Test					
		APL(cm)	LR(cm)	LD(mm)	APDM(g)	RDM(g)	DQI
Treatments	5	3.39*	2.30**	0.15**	0.00**	0.00 ^{ns}	4.47 ^{ns}
Means		4.66	6.49	1.58	0.00	0.00	0.00
CV%		11.47	13.37	9.69	28.50	24.66	23.79

** significant at 5% probability level; * significant at 1% probability; (ns) no significant difference

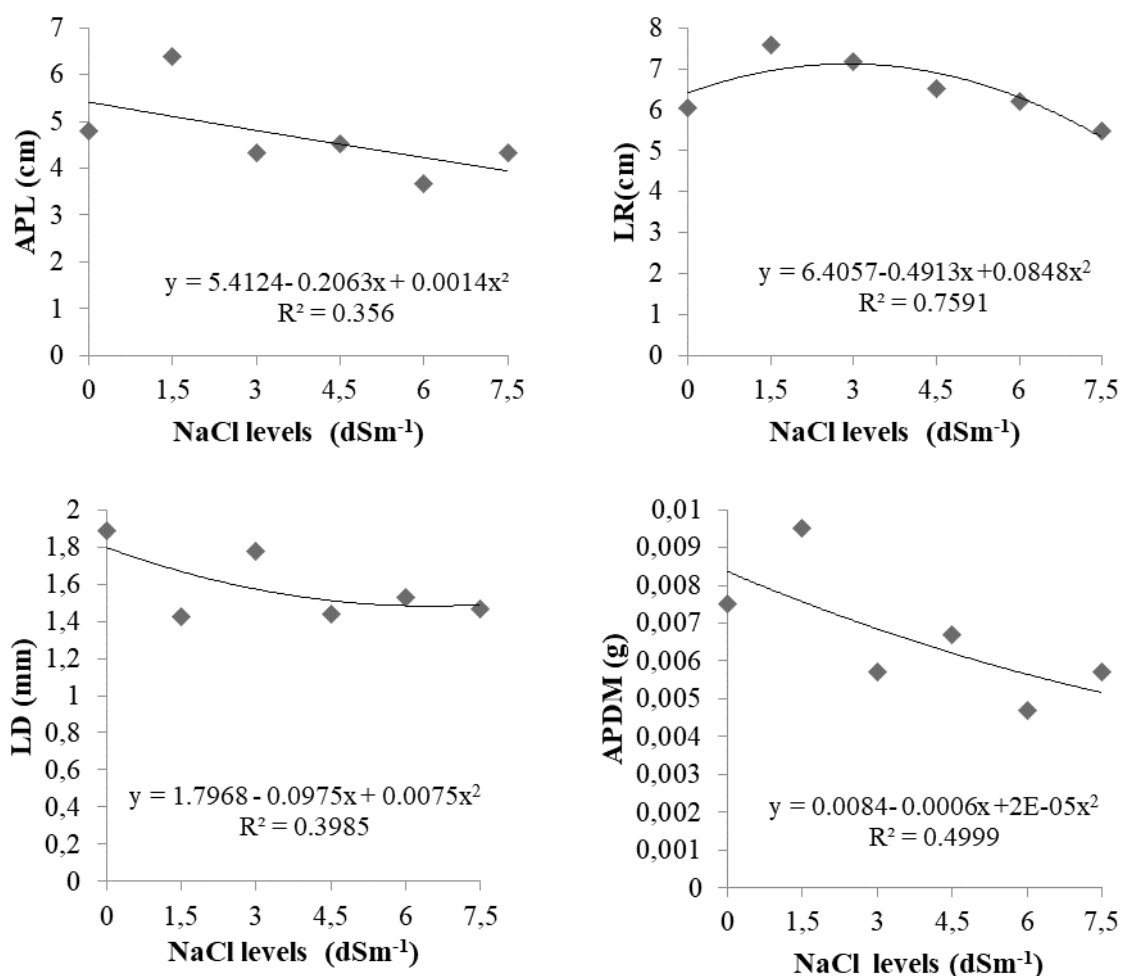


Figure 2. Aerial part length (CPA), root length (CPR), lap diameter (LD) and aerial part dry mass (MSPA) of *Cajanus cajan* seedlings germinated in different NaCl concentrations

Pedó *et al.* (2014), the lower allocation of dry matter mass may be a result of the effect of high sodium chloride concentration on hydrolysis mechanisms and mobilization of reserves for the seedling.

In relation to the emergence test, it can be seen in Table 2 that there was no significant difference among the NaCl concentrations for most of the analyzed variables. Only the first emergence count presented significant difference.

In Figure 3, it can be observed that from the concentration of 0.13 dSm⁻¹ NaCl, there was a reduction in the percentage of the first emergency count. Lima *et al.* (2015) observed that irrigation with saline water in *Albizia lebbek* (L.) Benth. caused a decrease of 95% on the emergence percentage, between 1.5 dSm⁻¹ and 4.5 dSm⁻¹ concentrations.

In Table 3 we can verify that there was no significant difference among the NaCl

concentrations for most of the variables analyzed in relation to vigor. For root dry mass and Dickson Quality Index, it was observed a significant difference among the treatments.

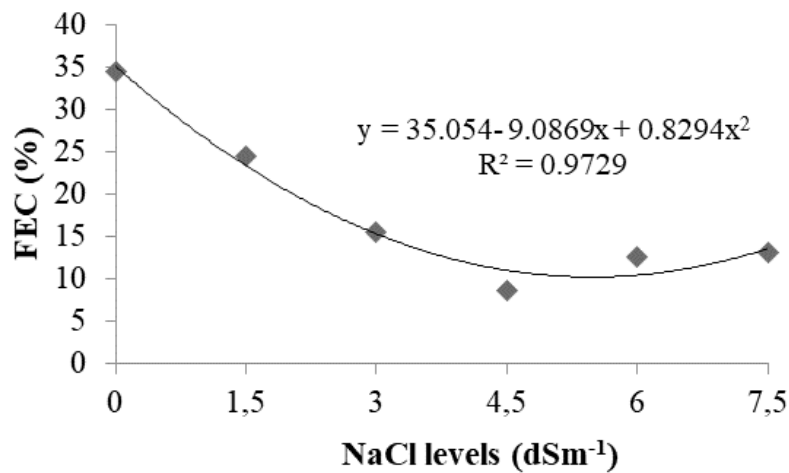
In Figure 4, it is possible to observe an inversely proportional behavior of the variables RDM and DQI with the increase in NaCl concentrations, showing a negative effect for Dickson.

In Figure 4, we can also observe that the values for dry mass of the root tend to increase with the increase in NaCl concentrations, reaching the minimum value at the concentration of 0.2 dSm⁻¹ and the maximum value at the concentration 7.5 dSm⁻¹. This fact can be explained by the tolerance of this species to salinity, beyond the fact that they are in the initial phase of germination, using the reserve of nutrients present in the seed. Schafranski *et al.* (2019), evaluated two bean species, *Phaseolus vulgaris* L., (black and carioca), and reported that

Table 2. Analysis of variance for emergence (E%), emergence speed index (ESI), first emergence count (FEC%), hard seeds (HS%), and dead seeds (DS%) of *Cajanus cajan*

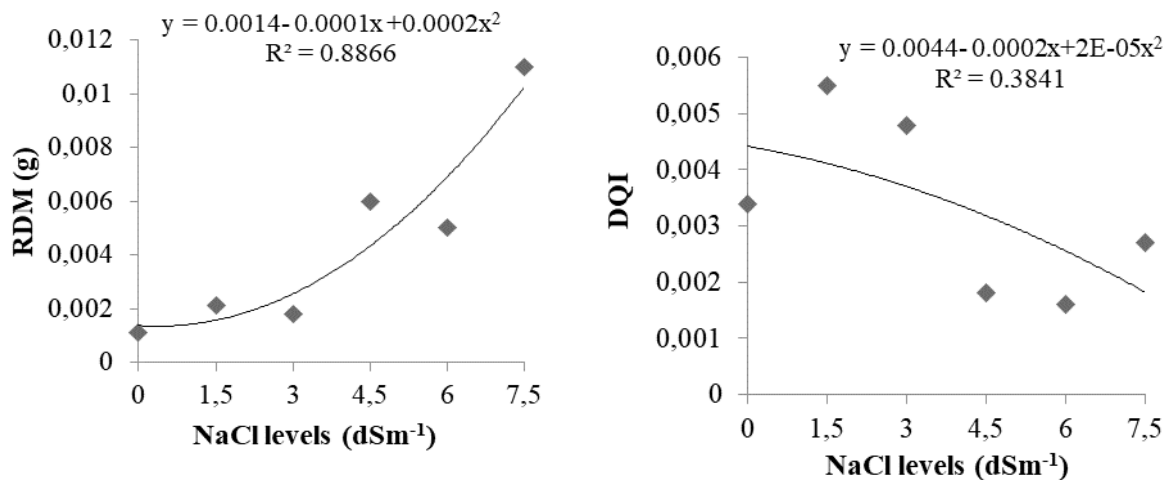
SV	F Test					
	DF	E%	ESI	FEC%	HS%	DS%
Treatments	5	116.0 ^{ns}	50.50 ^{ns}	372.9 ^{**}	1.60 ^{ns}	257.9 ^{ns}
Means		51.00	14.35	18.08	1.0	13.75
CV%		26.16	30.89	63.68	163.30	83.57

** significant at 5% probability level; (ns) no significant difference

**Figure 3.** First emergence count (FEC%) of *Cajanus cajan* in different NaCl concentrations**Table 3.** Summary of analysis of variance for aerial part length (APL), root length (RL), lap diameter (LD), aerial part dry mass (APDM), root dry mass (RDM), Dickson Quality Index (DQI) of *Cajanus cajan* seedlings

SV	DF	F Test					
		APL(cm)	RL(cm)	LD(mm)	APDM(g)	RDM(g)	DQI
Treatments	5	0.48 ^{ns}	0.79 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{**}	0.00 [*]
Means		1.67	3.75	1.37	0.00	0.01	0.00
CV%		27.47	25.73	7.88	138.76	63.49	45.17

** significant at 5% probability level; * significant at 1% probability; (ns) no significant difference

**Figure 4.** Root dry mass (RDM) and Dickson Quality Index (DQI) of *Cajanus cajan* seedlings submitted to different concentrations of NaCl

the effects of NaCl on the dry mass of seedlings were more intense for the commercial group carioca.

Gama *et al.* (2009) worked with common bean (*Phaseolus vulgaris* L.) under saline stress, and observed a reduction in bean biomass, indicating several growth limitations, besides affecting other morphological parameters, such as: height, number of leaves, root and aerial part length. Consequently, the weight of these variables reduced. In another species such as rice, Larré *et al.* (2011) evaluated the physiological quality of these seeds conditioned to saline solution in brassinosteroid, and stated that a reduction in dry mass could occur due to the reduction of carbon gain and energy expenditure for adaptation to salinity.

Considering the DQI variable, the values tend to decrease with the increase in NaCl concentrations, since it is based on the height and diameter ratio and on the allocation of biomass between root and aerial part. Sousa *et al.* (2017) observed in their research with *Talisia esculenta* (A. St.-Hil.) Radlk, that from the conductivity of 1.0 dSm⁻¹, regardless of the use of bovine biofertilizer, there was a reduction in the growth and quality of the seedlings from Python tree.

The DQI represents the quality of seedlings, which possibly provides an adequate development. For the *C. Cajan* species it demonstrates a certain fragility, due to the plasticity that the species has in relation to its total development as a future seedling and/or plant. Salinity can cause an imbalance in the concentration of Na⁺ and Cl⁻ ions inside the cells, exerting a toxic effect during seedling formation. Seeds, when exposed to salinity, undergo changes in their metabolism and even a reduction in germination and vigor (PINHEIRO *et al.*, 2013).

CONCLUSION

- The progressive addition of NaCl to the substrates does not cause a significant effect on the germination and emergence of *C. cajan* seedlings. However, it reduces the seedlings vigor under the tested conditions of the experiment.

AUTHORSHIP CONTRIBUTION STATEMENT

SANTOS, S. B. C.: Conceptualization, Data curation, Investigation, Project administration,

Writing – original draft; **RANGEL, I. S. L.:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing; **SOUZA, K. L.:** Data curation, Investigation; **DANTAS, A. C.:** Data curation, Investigation; **MEDEIROS, M. G.:** Data curation, Investigation; **NASCIMENTO, M. G. R.:** Supervision, Visualization.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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