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Phosphate fertilization improves vegetative growth and quality of pine cone seedlings

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Abstract

Adding phosphorus in the composition of the substrate for the seedlings production contributes to a faster growth of seedlings, increases the resistance to biotic and abiotic stresses, also improves the efficiency in the use of nutrients and water in many vegetal species. The goal of this research was to evaluate the effect of levels of simple superphosphate on the production of pine cone (*Annona squamosa* L.) seedlings. Six doses of single superphosphate (0.0 kg m^{-3}; 0.75 kg m^{-3}; 1.5 kg m^{-3}; 2.25 kg m^{-3}, 3.0 kg m^{-3} e 3.75 kg m^{-3} of substratum) were tested. A completely randomized design was used, with four replications and ten plants per plot. At 120 days after sowing, number of leaves, leaf area, shoot height, stem diameter, shoot dry mass, root dry mass, total dry mass, ratio between shoot dry mass and root dry mass, chlorophyll a, chlorophyll b, total chlorophyll, and Dickson's Quality Index were evaluated. More vigorous pine cone seedlings, with maximum growth, were noted with the single superphosphate application between 0.6 to 1.2 kg m^{-3} of substrate. The phosphate fertilizer favored the growth especially of the roots and leaf area and improved the quality of pine cone seedlings.

Keywords: *Annona squamosa*, seedlings production, simple superphosphate

Introduction

The cone pine (*Annona squamosa* L.) is a fruit tree from the Annonaceae family that presents a tropical and subtropical distribution, besides being one of the most cultivated species from the Annona genus with great economic significance in Brazil, mainly in the northeast and southeast regions. The socioeconomic importance of the crop has increased recently, especially in the semi-arid region, which, due to the warm climate, produces high quality fruits throughout the year (São José et al., 2014).

The culture can be propagated sexually or asexually, however, the production of seedlings through seeds is more widespread among growers and nurseries, due to the simplicity and speed of the method. Vegetative propagation is used only when one wants to have the characteristics of an elite selection or material, as well as the production of resistant rootstocks, with grafting being the most used (Ferreira et al., 2012).

The production of high quality seedlings is a major factor in the establishment of fruit trees and for the formation of orchards. However, factors such as the substrate, climate, nutritional and sanitary conditions, directly influence the development of seedlings (Nogueira et al., 2021).

Among the nutrients required for the development of the seedling, the supply of phosphorus must be pointed out, due to its low natural availability in tropical regions (Oliveira et al., 2015), in addition to the high demand during the initial growth period of perennial seedlings (Novais & Smyth, 1999). In this sense, the pine cone trees present a high nutritional demand, especially for P, when compared to other fruit trees (Cavalcante et al., 2012).

Phosphorus is considered an essential nutrient

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for the vegetal biocycle and has a key role in the photosynthetic, respiratory, energy storage and transfer processes (Daly et al., 2015). Besides, it helps in the formation of the root system, increases the tolerance to biotic and abiotic stresses, improves the water use efficiency and increases the active absorption of other nutrients (Taiz & Zeiger, 2017).

Studies have shown a beneficial effect of the phosphate fertilization on fruit tree seedlings: açai (Araújo et al., 2018), papaya (Souza et al., 2015), soursop (Soares et al., 2007). To produce pine cone seedlings Araújo et al. (1999) recommend 1.5 g dm^{-3} of simple superphosphate in substrate, while Moreira et al. (2019) claim that 350 mg dm^{-3} provides sufficient dry matter accumulation for pine cone seedlings. Freitas et al. (2013) suggest for the initial development of pine cone seedlings, the application of 10.83 mL dm^{-3} of Cosmofert®. Andrade et al. (2018) recommend the application of 2.24 g dm^{-3} of triple superphosphate.

The phosphorus required for optimum plant growth varies according to the species, besides they have differences in their capability of extracting the available forms of P (Abreu et al., 2005). The use of simple superphosphate has been highly recommended in order to supply phosphorus in plants, as it also contains calcium and sulfur in its chemical composition.

Therefore, the goal of this research was to evaluate the effect of levels of simple superphosphate on the production of pine cone (*Annona squamosa* L.) seedlings.

Material and Methods

The experiment was conducted in 2016, in a greenhouse (9 ° 19'35" S, 40 ° 32'53" O and 370 m altitude), in Federal University of São Francisco Valley, at Petrolina (PE), Brazilian semiarid region. The average climatic data of temperature and relative humidity, during the experiment, were 25.02 °C and 56.77%, respectively.

A completely randomized design was used, with four replications and ten plants per plot. Six doses of single superphosphate (0.0 kg m^{-3}; 0.75 kg m^{-3}; 1.5 kg m^{-3}; 2.25 kg m^{-3}, 3.0 kg m^{-3} e 3.75 kg m^{-3} of substratum) were tested.

For the production of seedlings, an organic substrate of enriched sugarcane bagasse was used, which is a mixture of sugarcane bagasse and filter cake (0.84% of N; 0.65% of P; 0.9% K; 2.283% Ca; 0.39% Mg; 0.32% S; 25.2% organic matter; 17/1 of C / N ratio and 6.1 pH in H_20). The simple superphosphate (18% P_2O_5, 16% Ca and 8% S) was used as a source of phosphorus. The mixture of substrate and phosphate fertilizer was placed in black polyethylene bags with 1 dm³ volume.

Pine cone seeds obtained from ripe fruits with similar size and similar peel aspect undergone soaking for 24 hours, subsequently drying in the shade for another 24 hours. Sowing was carried out by placing 3 seeds per container (bag), approximately 0.02 m depth. The seedlings were irrigated daily in a sufficient quantity to drain.

At 45 days after sowing (DAS), the percentage of germination (G) was estimated and the roughing was carried out, so that one plant remained per recipient. At 120 DAS, were evaluated: the number of leaves (NL), shoot height (SH), stem diameter (SD), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), A-chlorophyll content (A-Chl), B-chlorophyll content (B-Chl) and total chlorophyll (T-Chl).

The number of leaves was determined by counting the number of leaves. To measure SH and SD, a 60-centimeter-long ruler with 0.1 cm of precision and a digital caliper with 0.01 mm precision were used, respectively. To obtain SDM and RDM, the plants were dried in a forced air oven at a temperature of 60 °C, until they reached a constant mass, subsequently weighed on a precision scale (0.0001 g). The chlorophyll data were collected using a chlorophyll meter (Chlorofilog®, model-CFL1030) and the readings expressed in Falker Chlorophyll Index (FCI).

The predetermined growth characteristics were used in order to obtain the ratio between the shoot dry mass and the root dry mass (SDM.RDM^{-1}), and the Dickson's Quality Index (IQD). According to Dickson et al. (1960), growth characteristics are related for its calculation, as per the equation 1.

$$DQI = MST \div [(H \div DC) + (SDM \div RDM)] \quad (1)$$

Where: DQI = Dickson's quality index (g mm cm^{-1}); TDM = total dry mass (g); SH = shoot height (cm); SD = stem diameter (mm); SDM = shoot dry mass (g); and RDM = root dry mass (g).

The data were submitted to analysis of variance, through the F test ($p < 0.05$); when significant, polynomial regression equations were adjusted according to the applied levels of simple superphosphate.

Results and Discussion

The summary of analysis of variance for characteristics of physiological quality, seedling growth, and seedling quality is presented in Table 1. The results obtained revealed that phosphate fertilization, using the simple superphosphate as source of phosphorous, have significantly influenced all the characteristics of physiological quality, seedling growth, and seedling quality, except the shoot dry mass (SDM), with average value of 0,77 g seedling^{-1}. The variables showed a quadratic polynomial response when observed the simple superphosphate levels applied in mixture.

Table 1. Summary of variance analyses for studied variables, as a function of simple superphosphate levels applied to an organic substrate of enriched sugarcane bagasse, for the production of pine cone seedlings.

SV	DF	PG (%)	A-Chl	B-Chl	T-Chl	SH (cm)	SD (mm)	NL (leaf)	SDM (g)	RDM (g)	TDM (g)	SDM RDM^{-1} (g g^{-1})	DQI (g mm cm^{-1})
Dose	5	1672.96*	79.22*	17.66*	156.55*	24.27*	1.48*	11.13*	0.03^{ns}	0.08*	0.21*	0.49*	0.012*
Residue	18	68.52	13.38	2.90	11.12	1.17	0.07	0.60	0.01	0.01	0.04	0.070	0.002
VC (%)		14.68	13.56	26.46	9.98	11.42	11.18	11.33	15.58	19.72	15.85	15.84	17.72
Average		56.39	26.98	6.44	33.41	9.49	2.37	6.84	0.77	0.50	1.27	1.64	0.23

^{ns} no significant * significant at the level of 5% by F test.

The germination of pine cone seeds occurred between 15 and 20 days after sowing. (Figure 1). levels of simple superphosphate higher than 0.93 kg m^{-3} negatively affected the germination process of seeds. High contents of fertilizers and the prolonged period of contact with seeds can affect the physiological quality of seeds. Taiz & Zeiger (2017) have affirmed that. besides limitations on absorption of water by seed. the salt causes more significant damages since it penetrates the seed that is in contact with the solution containing it. Thus. the damage to seeds is not exclusively the result of reduction on the hydric potential induced by action of an osmotic agent. but also from direct contact with the salt. reducing the potential of seeds germination.

The *a* and *b* chlorophyll content. and also the total chlorophyll. increased reaching the maximum values in levels of simple superphosphate mixture of 1.18; 1.26 and 1.32 kg m^{-3}. respectively (Figure 2).

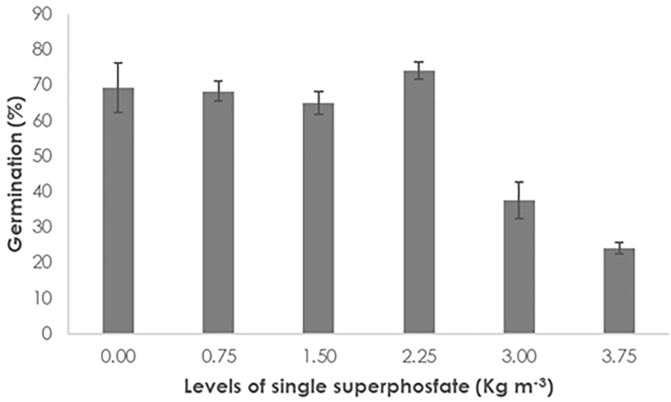

Figure 1. Levels of simple superphosphate and their effect on germination of pine cone seedlings
^{ns} no significant * significant at the level of 5%.

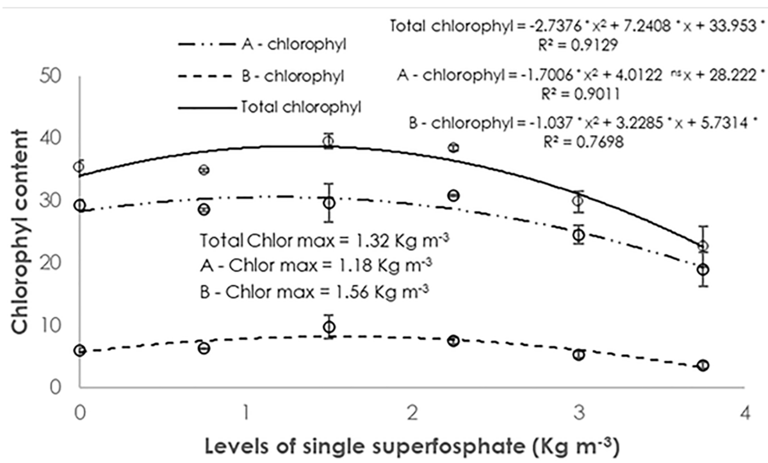

Figure 2. Levels of simple superphosphate and their effect on A-chlorophyll content (A). B-chlorophyll content (B) and total chlorophyll content (C) of pine cone seedlings.
^{ns} no significant * significant at the level of 5%

Silva et al. (2016) reported that the chlorophyll content in pigeonpea. increased with phosphorous levels and the phosphate fertilization allowed the increase of nitrogen levels on leaf tissues. advantaging the chlorophyll synthesis. In coffee plants. the cultivation of seedlings for 70 days fertilized with 572 mg kg^{-1} of P_2O_5 improved the photosynthetic performance influenced by higher levels of *a*. *b* and total chlorophyll (Silva et al., 2010).

Chlorophyll is the basic unit of plant energy systems during the process of photosynthesis. Higher levels of chlorophyll can be observed in balanced doses of phosphate fertilizer because the inner membrane of the chloroplast is impermeable to most phosphorylated compounds. where these compounds are used in photophosphorylation reactions. Decreases in P. especially in developing plants. can affect photosynthesis or respiration. leading to decreases in consumption and production of ATP and NADPH (Wietrzynski & Enge. 2021).

The content increasing of chlorophyll in leaves according to the phosphate fertilization suppling. is due to the higher absorption of nitrogen by the plant in presence of phosphorous. once there is a correlation between the chlorophyll content and the nitrogen concentration in leaves (Taiz & Zeiger. 2017).

The pine cone shoot height achieves the maximum value with the simple superphosphate dose at 0.69 kg m^{-3} (Figure 3A). the stem diameter with 1.14 kg m^{-3} (Figure 3B). the number of leaves with 1.05 kg m^{-3} (Figure 3C). the root dry mass with 1.16 kg m^{-3} (Figure 3D) and the total dry mass with 1.13 kg m^{-3} (Figure 3E).

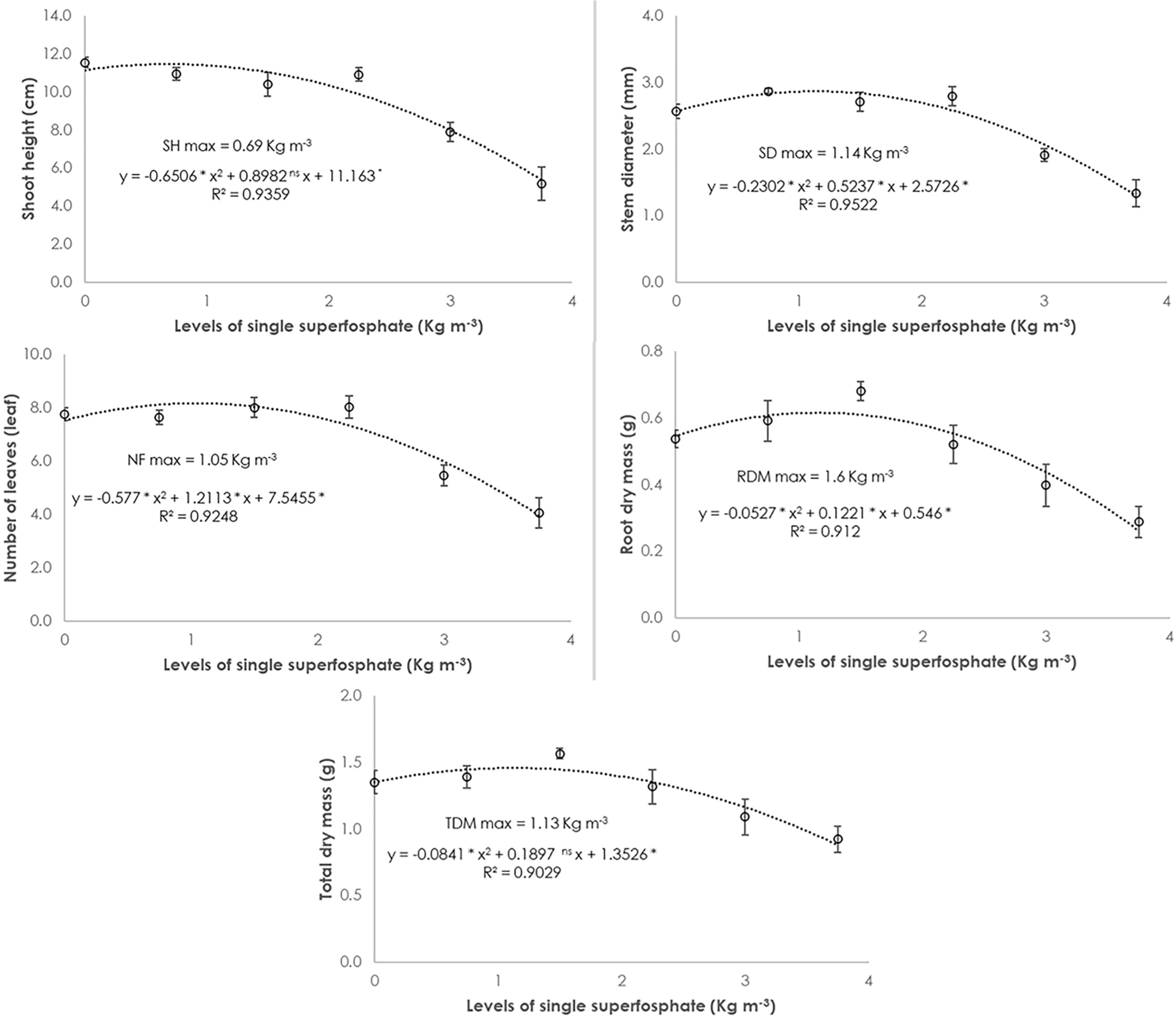

Figure 3. Levels of simple superphosphate and their effect on shoot height. stem diameter. number of leaves. root dry mass and total dry mass (E) of pine cone seedlings.

^{ns} no significant * significant at the level of 5%.

The dose of simple superphosphate that allowed the maximum growth. promoted an increase of 5% in the shoot height of pine cone seedlings and 12% of the stem diameter. The number of leaves increased 9%. and the root dry mass and the total dry mass. showed gains of 13% and 8%. respectively. by the same comparison.

The phosphorus addition to the substrate had a positive effect on the seedlings. despite the low increase of the growth characteristics analyzed. The increase was more significant in the root dry matter production. due to the importance of phosphorous in the formation of the root system of plants. A well-developed root system increases the efficiency of water and nutrient absorption (Lima et al., 2011) and provides better seedling fixation (Araújo et al., 2018). which favors the initial development of the plant in the field.

The optimum growth of pine cone seedlings was obtained with the addition of simple superphosphate in the range of 0.6 to 1.2 kg m^{-3}. The positive effect of phosphorus addition on the growth of pine cone seedlings are described in other studies (Andrade et al., 2018; Freitas et al., 2013; Moreira et al., 2019) as well as many other species such *Annona muricata* (Graviola. a Brazilian native fruit) (Soares et al., 2007); *Euterpe oleracea* (Açaí. a Brazilian native fruit) (Araújo et al., 2018); papaya (Souza et al., 2015) and Jatropha (Lima et al., 2011).

When the morphological growth characteristics are related. they can also provide relevant information about the seedling quality. According Gomes and Paiva (2012). the relation shoot dry mass and root dry mass (SDM. RDM^{-1}). equivalent to 2 is an indicative of seedlings with high quality. The simple superphosphate dose that provided the relation of SDM. RDM^{-1} equal to 2 was 3.35 kg m^{-3} (Figure 4).

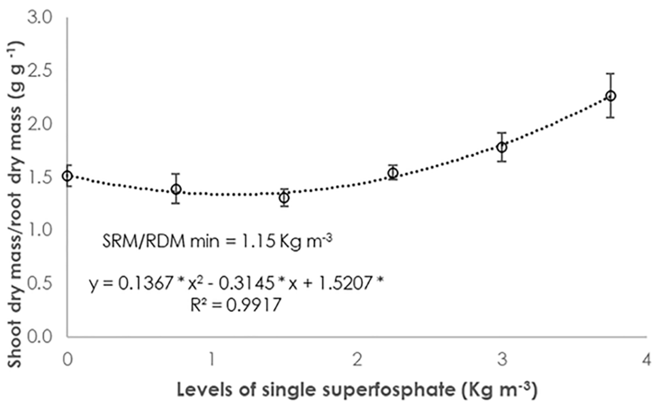

Figure 4. Levels of simple superphosphate and their effect on the ratio between shoot dry mass and root dry mass (SDM. RDM-1) of pine cone seedlings.
ns **no** significant * significant at the level of 5%**.**

Seedlings with higher dickson´s quality index (DQI) present better quality. once they have robustness and balance in the dry mass distribution allowing higher capacity od adaptation. development and surviving in the field (Araújo et al., 2018). being the DQI superior to 2. an indicative of seedlings with quality (Silva et al., 2019).

The DQI of pine cone seedlings achieved maximum value (0.28) using the simple superphosphate dose of 1.34 kg m^{-3} (Figure 5). When observed the maximum value of dose used. the DQI increased 27%. when in comparison to the substrate without addition of phosphorous.

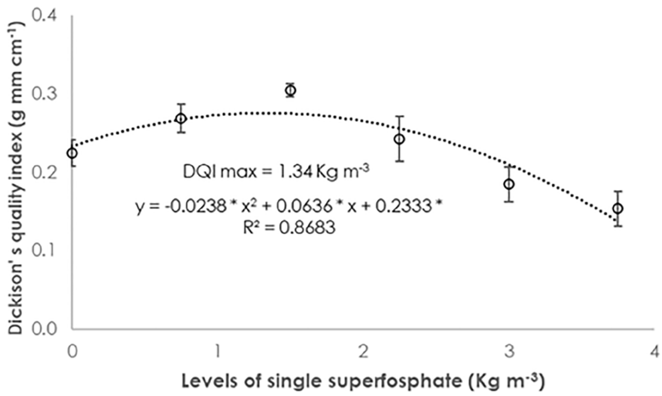

Figure 5. Levels of simple superphosphate and their effect on Dickson´s quality index (DQI) of pine cone seedlings.
ns no significant * significant at the level of 5%.

Studies have identified an increase in DQI values of pine cone seedlings and other species. when applying some source of phosphorus into the substrate. as observed to pine cone (Freitas et al., 2013), *soursop* (Soares et al., 2007), *açaí* (Araújo et al., 2018) and papaya (Souza et al., 2015).

To produce seedlings with high quality it is important to know the effect of the macronutrient phosphorous. since in addition to promoting the growth and development of roots. phosphorous increase the efficiency of water used by plants. and also plays a fundamental key in photosynthetic. respiratory and energy storage and transfer processes.

On the other hand. high levels of phosphorus had a negative effect on the physiological and growth characteristics of the pine cone seedlings. with reduction of the chlorophyll content. shoot height. stem diameter. number of leaves. and on total dry mass. which impairs on the quality of the seedlings.

High levels of phosphorus in the substrate may affect the availability of other nutrients and. consequently. harm the plant development (Macedo & Teixeira 2012). In addition. crops present a huge difference in their capability of extracting available forms of phosphorus from the substrate (Abreu et al., 2005).

Therefore. the dose that promotes higher plant growth and better quality of seedlings. has a wide range of variation. since this value depends on specific characteristics of the species and the substrate used. in which chemical and physical attributes compromise on the behavior of phosphorus and its absorption by plant roots.

Conclusions

The maximum pine cone growth was observed adding low levels of simple superphosphate on estimated levels ranged to 0.6 to 1.2 kg m^{-3}. The phosphate fertilizer allowed specially the roots growth and the addition of phosphorus increased the seedling quality in 27%.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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