

Initial growth of chia (*Salvia hispanica* L.) submitted to nitrogen, phosphorus and potassium fertilization

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Abstract

Research results on agronomic techniques for chia are still few, although this culture is very widespread. The study was carried out under greenhouse conditions, from June to August 2016, in the city of Campina Grande, PB, Brazil, with geographic coordinates 7°13'11" S, 35°53'31" W and altitude of 547.56 m. During this period the average air temperatures inside the greenhouse varied between 23.7 °C and 33.8 °C, with mean relative humidity around 52%. The objective of this study was to investigate the effects of nitrogen, phosphorus and potassium fertilization application on the development of chia. The research consisted of three independent experiments, following a completely randomized design to evaluate the performance of nitrogen (N), phosphorus (P) and potassium (K) in six doses, with three replications. Each experimental unit consisted of a plastic vase filled with 8 kg of sandy soil. The nutrient doses were as follows: 0; 25; 50; 75; 100 and 125 kg ha⁻¹ of N; 0; 20; 40; 60; 80 and 100 kg ha⁻¹ of P and 0; 20; 40; 60; 80 and 100 kg ha⁻¹ of K. Analysis was made of the height of the plants (30 and 60 DAS) (measured with a graduated ruler), number of leaves and of inflorescence per plant and weight of the dry biomass. First results indicate that the nitrogen, phosphorus and potassium doses favored the growth of chia plants in relation to control promoting an increase in all studied variables, evidencing the necessity of cultivation by these elements. In general, considering the values of the variables according to the increasing doses of nutrients, a preliminary recommendation could be indicated, under the conditions of this research, as 125: 100: 40 kg ha⁻¹ of N: P: K. So the effect of these nutrients on chia need to be more investigated.

Keywords: *Salvia hispanica* L.; mineral fertilizers; Development of culture.

Abbreviations: N_nitrogen; P_phosphorus; K_potassium; DAS_ days after sowing; LA_ linoleic acid.

Introduction

The growing concern of modern society to have a healthy diet has contributed to the increase in the demand for foods that are rich in nutrients and that contain, in this way, greater amounts of beneficial substances to the organism. The nutritional properties of chia seed make it an excellent source of important substances such as Omega-3, fiber and calcium, making it more and more recommended by experts as a complement in the food menu. Chia (*Salvia hispanica* L.) is an annual herbaceous species belonging to the Labiatae family. Domesticated in Mesoamerica around 2600 B.C. it has been a staple food in Mexico between 1500 and 900 B.C. (Poza, 2010). In the Pre-Columbian times, it was one of the four main basic foods used by the civilizations that inhabited Central America, behind only corn and beans, but more prominently than other crops such as amaranth (Ayerza and Coates, 2005). The main interest in this seed is the content of polyunsaturated fatty acids, alpha-linolenic acid (18: 3 n-3, LNA) and linoleic acid (18: 2 n-6, LA) (Ayerza, 2009; Mohd et al., 2012), since these compounds support immune functions, inhibit the proliferation of proinflammatory lymphocytes and cytokines, act to prevent the incidence of cardiovascular diseases, and maintain the integrity of cell membranes and neurotransmitters (Yao et al., 2012; Ludwig et al., 2013). Currently chia seeds are grown commercially in Australia, Bolivia, Colombia, Guatemala, Mexico, Peru and Argentina, with emphasis on the provinces of Salta, Jujuy,

Tucumán and Catamarca (Busilacchi et al., 2013). In Brazil, the regions of western of Paraná and northwest of Rio Grande do Sul began to invest in chia cultivation in the last harvests, presenting good results, despite the lack of information regarding the nutritional requirements of the plant (Migliavacca et al., 2014). Ayerza and Coates (2009) have studied the crop in different areas in Central America, but research results on agronomic techniques for chia are still few (Bochicchio et al., 2015b). The fertilization recommendations are based on studies done in other countries with different climatic and edaphic conditions. In Argentina, for example, between 15 and 45 kg of nitrogen and 37 kg of phosphorus per hectare are used, while in Mexico, 68 kg of nitrogen per hectare (Ayerza and Coates, 2006), however, recent crops already use quantities greater than 100 kg ha⁻¹ of nitrogen (Poza, 2010). Cover fertilization is carried out in installations at three different times, at 30, 60 and 90 days after sowing, using balanced formulations such as NPK fertilizer 15-15-15 (Miranda, 2012). Efficient fertilization is important in both economic and environmental terms. This minimizes nutrient losses to the environment while yielding optimum crop yields. The quantities and proportions required of different nutrients, for each crop and each soil in particular, must be respected. The nutrients nitrogen, phosphorus and potassium are constituents of many plant components, such as proteins, nucleic acids and chlorophyll, and are essential for processes

such as energy transfer, maintenance of internal pressure and enzymatic action. Therefore, it is necessary to generate local information, through studies, that guarantee an efficient fertilization, with reduction of costs and prevention of negative environmental impacts. Considering the importance of this culture, the lack of information about the fertilization recommendation, mainly for the Brazilian Northeast region, this work had as objective to evaluate the effect of different doses of nitrogen, phosphorus and potassium on the initial development of chia plants.

Results and Discussion

Effect of N doses in plant height, leaves and inflorescence number and dry biomass yield.

Chia seedlings emerged three DAS and had slow initial growth, which was also verified by Waisle (2013). Regarding the effect of the nitrogen on the development of the chia, the average values for plant height at 30 and 60 DAS, number of leaves, inflorescence number and dry biomass showed significant differences at 1% of probability with nitrogen fertilization (Table 1). According to Coates and Ayerza (1996), applying 100 kg ha^{-1} of nitrogen fertilizer in the chia crop, the highest production of seeds was obtained with fertilizer N:P:K 40:00:00 in relation to 20:00:00 showing the importance of N in this culture.

Nitrogen is the nutrient most required by crops, as it is a chemical element in the structure of amino acid, protein, enzyme, pigment and by-product molecules (Malavolta et al., 1997), which perform functions related to plant growth and development. Probably because of this, the plants that did not receive nitrogen had lower heights (17.63 and 37.33 cm at 30 and 60 DAS, respectively) than the others with increasing doses of this element (Table 1). At 30 and 60 DAS the height of the plants increased as the nitrogen doses reached with the highest dose 26.21 cm and 61.08 cm, respectively. In both cases, the values fit the linear regression (Fig.1) disagreeing Frutos et al. (2014), once the authors noted that the addition of nitrogen to the soil (0; 40; 80 and 120 kg ha^{-1}) did not have a significant effect on the plant height and number of branches of chia plants, which varied between 59 and 66 cm, and 7.9 and 9.3, respectively.

The mean height at 60 DAS was 51 cm (Table 1) result lower than 62; 63 and 112 cm observed by Busilacchi et al. (2013), Frutos et al. (2014) and Hernández and Miranda (2008), respectively. In the present work, the lower height of the plants should be related to the fact that the plants do not reach the total development according to the plants observed by these other authors. The nitrogen doses influenced the number of leaves and of inflorescence per plant, and those values fit the quadratic regression with a maximum point at $103 \text{ kg ha}^{-1} \text{ N}$ (18 leaves) and $79 \text{ kg ha}^{-1} \text{ N}$ (10 inflorescence), respectively (Fig. 2A and 2B). In the case of dry biomass, the values increase according to the increase of the nitrogen doses reached with the highest dose ($125 \text{ kg ha}^{-1} \text{ N}$) 8.06 g (Table 1) which fit the linear regression (Fig. 3). In general the response of chia to nitrogen is reported to be controversial (Kartzow, 2013) since Bochicchio et al. (2015a) studying the effect of agricultural management on leaf and fruit production of chia without and with N (20 kg ha^{-1}) noted the lack of response to N top-dressing for leaf area index and biomass.

Effect of P doses in plant height, leaves and inflorescence number and dry biomass yield.

The adequate supply of P is essential since the initial stages of plant growth. According to Grant et al. (2001), the limitations on the availability of P at the beginning of the vegetative cycle may result in developmental constraints, from which the plant does not recover later even by increasing the P supply at appropriate levels. Deficiency can also reduce the synthesis of nucleic acid and protein, inducing accumulation of soluble (N) nitrogen compounds in the tissue and may slow the growth of the cell. Therefore, P deficiency symptoms include a decrease in plant height, delay in emergence of leaves and reduction in sprouting and development of secondary roots, dry matter production and seed production. In this research phosphate fertilization significantly influenced the height of the plants at 30 DAS, the number of leaves and the number of inflorescences at 1% probability level; and at the 5% probability level, influenced plant height at 60 DAS and dry biomass (Table 1).

At 30 DAS the height of the plants increased as the phosphorus doses reached with the highest dose ($100 \text{ kg ha}^{-1} \text{ P}$) 22.49 cm, ie, the values fit the linear regression. At 60 DAS the values of height of the plant fit the quadratic regression with a maximum point at $68.72 \text{ kg ha}^{-1} \text{ P}$ (22.19 cm) (Fig. 1).

The phosphorus doses influenced the number of leaves per plant and the dry biomass and those values fit the quadratic regression with a maximum point at $121 \text{ kg ha}^{-1} \text{ P}$ (22 leaves) and $71.25 \text{ kg ha}^{-1} \text{ P}$ (6.18 g), respectively (Fig. 2A and 3). In the case of number of inflorescence, the values increase according to the increase of the phosphorus doses reached with the highest dose ($100 \text{ kg ha}^{-1} \text{ P}$) 8.58 inflorescence (Table 1) which fit the linear regression (Fig. 2B).

The plants cultivated without phosphorus application had the lowest values of plant height (17.63 and 37.33 cm at 30 and 60 DAS, respectively), number of leaves (7.67), number of inflorescence (5.17) and dry biomass (3.52 g) compared to increasing doses of phosphorus (Table 1). This is because the availability of P favored the growth of the plant, promoted greater emission and growth of leaves and inflorescence of the crop, consequently, greater capture of solar radiation and increase in the production of photoassimilates (Bonfim-Silva et al., 2011). According to Fernandez and Ascenio (1994) and García-Sánchez et al. (1996), phosphorus deficiency is reported as limiting in the dry matter production of plants.

Effect of K doses in plant height, leaves and inflorescence number and dry biomass yield.

Plant height at 30 and 60 DAS was significantly influenced at the 5% probability level with increasing doses of potassium; the other parameters evaluated varied at 1% probability (Table 1). Potassium is involved in most of the biological processes in a plant and when not available at the minimum dose can reduce crop development and consequently productivity (Malavolta et al., 1997, Castro and Oliveira, 2005). Therefore, the plants that were not fertilized with potassium had the lowest plant height, less amount of leaves and inflorescence and lower weight of dry biomass.

At 30 DAS the height of the plants increased as the potassium doses reached with the highest dose ($100 \text{ kg ha}^{-1} \text{ K}$) 22.42 cm, ie, the values fit the linear regression. At 60 DAS the values of height of the plant fit the quadratic regression with a maximum point at $67.95 \text{ kg ha}^{-1} \text{ K}$ (61.81 cm) (Fig. 1).

Table 1. Summary of the analyses of variance for the plant height, leaves number, inflorescence number and dry biomass of the aerial of the chia, general average values and averages depending on the treatments with the use of nitrogen, phosphorus and potassium.

Source of Variation	DF	Mean squares				
		Plant height		LN	IN	DB
		30 DAS	60 DAS			
Nitrogen	5	23.11 **	270.59 **	46.59 **	9.39 **	7.97 **
Linear	1	66.25 **	1268.87**	145.83 **	22.67 **	37.95 **
Quadratic	1	0.45 ^{ns}	54.79 ^{ns}	27.34*	20.00 **	1.26 ^{ns}
Mean		21.80 cm	51.06 cm	15.28	8.61	5.39 g
CV(%)		9.03	9.91	11.44	12.24	11.55
Treatments, kg ha ⁻¹		Mean				
0		17.63 b	37.33 c	7.67 b	5.17 b	3.52 d
25		22.73 ab	41.77 bc	16.00 a	8.83 a	4.13 cd
50		21.08 ab	52.20 ab	16.33 a	8.83 a	4.83 bcd
75		21.83 ab	55.57 ab	16.33 a	10.17 a	5.57 bc
100		21.28 ab	58.42 a	16.00 a	9.67 a	6.23 b
125		26.22 a	61.08 a	19.33 a	9.00 a	8.06 a
Phosphorus	5	34.20 *	293.72 **	43.59 *	21.39 *	3.38 **
Linear	1	108.07**	657.38 **	150.03 **	94.00 **	4.29 **
Quadratic	1	1.21 ^{ns}	394.75 **	45.01 *	0.03 ^{ns}	3.38 **
Mean		22.49 cm	56.35 cm	14.08	8.58	5.45 g
CV (%)		12.92	10.06	21.61	24.29	8.86
Treatments, kg ha ⁻¹		Mean				
0		17.63 b	37.33 b	7.67 b	5.17 b	3.52 b
20		22.45 ab	57.00 a	11.33 ab	6.50 ab	6.44 a
40		22.83 ab	64.28 a	16.33 a	8.67 ab	5.24 a
60		21.92 ab	59.87 a	17.00 a	7.83 ab	6.18 a
80		21.88 ab	56.03 a	15.00 ab	11.83 a	5.30 a
100		28.20 a	63.57 a	17.17 a	11.50 a	6.02 a
Potassium	5	26.21 *	235.85 *	41.41 **	8.08 **	2.27 **
Linear	1	115.66**	541.93 **	113.67**	6.34 *	4.89 **
Quadratic	1	12.42 ^{ns}	342.65 *	60.52 **	22.02 **	4.27 **
Mean		22.42 cm	55.36 cm	14.36	8.30	5.28 g
CV (%)		12.61	12.87	10.79	13.54	11.15
Treatments, kg ha ⁻¹		Mean				
0		17.63 a	37.33 b	7.67 c	5.17 b	3.52 b
20		20.03 a	59.20 a	12.67 b	9.67 a	5.54 a
40		23.30 a	58.17 a	17.67 a	8.83 a	5.67 a
60		23.90 a	59.03 a	16.33 ab	9.50 a	5.81 a
80		24.40 a	58.12 a	15.00 ab	8.50 a	5.46 a
100		25.28 a	60.30 a	16.83 ab	8.17 ab	5.68 a

DF= Degree of Freedom, ^{ns}, * and ** no significant, significant at 5 and 1% level, respectively. CV = Coefficient Variation; LN = leaves number of; IN = inflorescence number; DB= Dry biomass of aerial part.

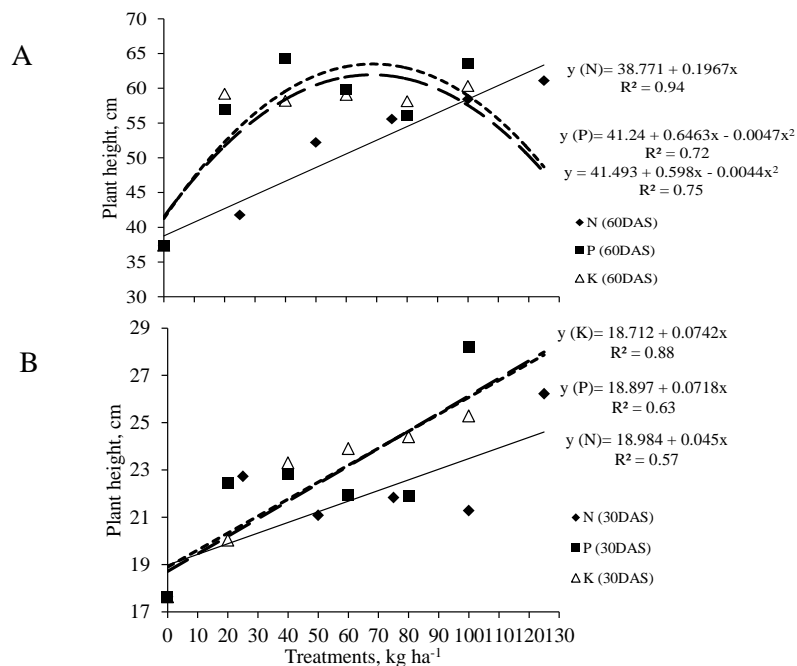


Fig 1. Plant height depending on nitrogen (—), phosphorus (---) and potassium (---) doses at 60 (A) and 30 (B) days after sowing (DAS) in chia cultivation.

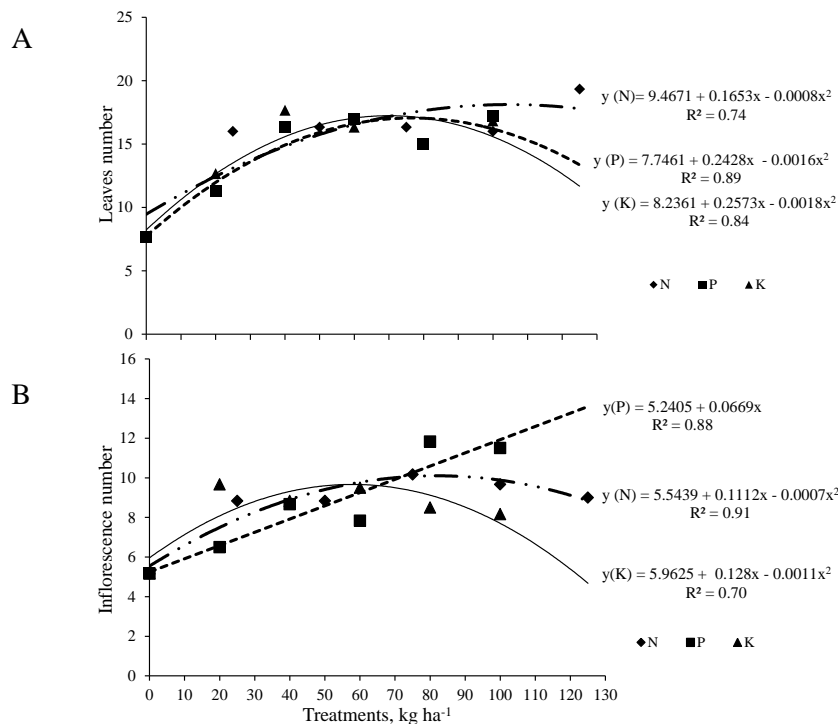


Fig 2. Leaves number (A) and inflorescence number (B) depending on nitrogen, phosphorus and potassium in chia cultivation.

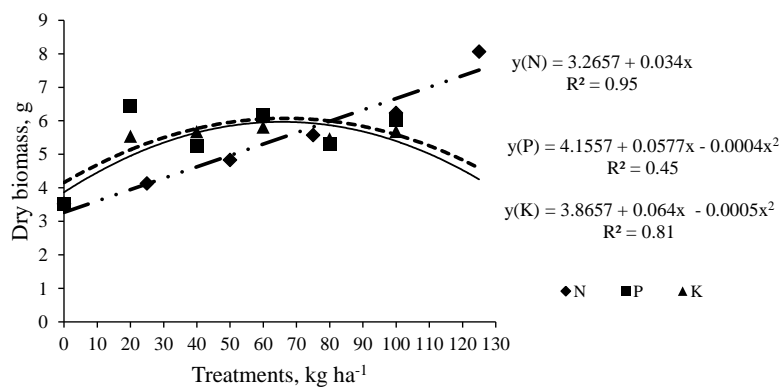


Fig 3. Dry biomass depending on nitrogen, phosphorus and potassium doses in chia cultivation.

The potassium doses influenced the number of leaves and number of inflorescence per plant and the dry biomass and those values fit the quadratic regression with a maximum point at 128 kg ha⁻¹ K (24 leaves), 64 kg ha⁻¹ K (10 inflorescence) and 64 kg ha⁻¹ K (5.91 g), respectively (Fig. 2A; 2B and 3). It is interesting to observe that in some studied variables, among the treatments with N, P and K, there were no relevant differences in the results since there was a significant difference only between the control treatment in relation to the others treatments. For example, the numbers of leaves and inflorescences were influenced by the application of nitrogen only in relation to the plants not fertilized (control treatment). There was no significant difference between increasing nitrogen doses. The same can be observed at plant height at 60 DAS and dry matter in relation to phosphorus and plant height at 30 DAS and dry matter in relation to potassium. The present study provides evidence that nitrogen, phosphorus and potassium fertilization affect the chia height, the number leaves and inflorescence and plant dry biomass. However, the plants were harvested at 60 DAS before reaching maturation, seed

production period. This is because in the period from 40 to 60 DAS there were some problems such as: the plants began to dry with symptoms like burning in the leaves. As research on chia cultivation is few, without information on levels of fertilization, on symptoms of deficiency and / or toxicity and diseases, it was not possible to solve such problems. Probably, factors intrinsic to the environment, like high temperatures, mainly inside the greenhouse, can have alter the response of plants to N, P and K fertilization, since there was no water stress in the plants. It is therefore important to continue this research by installing experiments at different times with milder temperatures. Anyway, the research has its merit because it is the beginning of so many that will be made based on this initial data.

Materials and Methods

Plant material and location of the experiment

The experiment was carried out under greenhouse conditions, from June to August 2016 at the Agricultural Engineering

Department of the Federal University of Campina Grande, Paraíba State, Brazil located in the municipality of Campina Grande, Paraíba State with geographic coordinates 7°13'11'' S, 35°53'31'' W and altitude of 547.56 m. During this period the average air temperatures inside the greenhouse varied between 23.7 °C and 33.8 °C, with mean relative humidity around 52%.

Experimental design and treatments

The research consisted of three independent experiments, following a completely randomized design to evaluate the performance of nitrogen (N), phosphorus (P) and potassium (K) in six doses, with three replications, totaling 18 experimental units for each nutrient. The nutrient doses were as follows: 0; 25; 50; 75; 100 and 125 kg ha⁻¹ of N; 0; 20; 40; 60; 80 and 100 kg ha⁻¹ of P and 0; 20; 40; 60; 80 and 100 kg ha⁻¹ of K. Each experimental unit with treatments of N, was fertilized with 60:60 kg ha⁻¹ of P:K; each experimental unit with treatments of P or K, was fertilized with 75:60 kg ha⁻¹ of N:K and 75:60 kg ha⁻¹ of N:P, respectively. These amounts of N, P and K were determined for greenhouse experiments according to Novais et al. (1991). The N sources used were urea (45% of N) being applied 30 days after sowing (DAS) in cover fertilization, soon after thinning, when there were only two plants per pot. Potassium (potassium chloride) and phosphorus (simple superphosphate) were totally applied on foundations, at the dosages corresponding to the treatments.

Conduct of the study

Each experimental unit consisted of a plastic vase filled with 8 kg of soil with the following chemical and physical characteristics according to the methodology of Embrapa (1997): pH (H₂O) = 5.56; Ca = 2.93 cmol_c kg⁻¹; Mg = 2.26 cmol_c kg⁻¹; Na = 0.10 cmol_c kg⁻¹; K = 0.25 cmol_c kg⁻¹; H + Al = 3.74 cmol_c kg⁻¹; P = 18.4 mg kg⁻¹; OM = 14.8 g kg⁻¹; sand = 832 g kg⁻¹; silt = 81.4 g kg⁻¹; clay = 86.6 g kg⁻¹.

In order to ensure a uniform germination of the seeds of chia, on the soil, in each experimental unit was placed 150 g of commercial substrate. Then twenty-five seeds of chia (*Salvia hispanica* L.) were sown directly in the pots at a 2 cm depth. Thirty days after sowing (DAS), seedlings were thinned to two plant per pot and 40 DAS, leaf fertilizer based on micronutrients was applied in the concentration of 1g L⁻¹ of water. The plants were irrigated daily, with the volume of water needed to reach the soil field capacity. The water content in the soil was monitored by weighing the experimental units, and irrigation was performed manually using rainwater with an electric conductivity of 0.02 dS m⁻¹.

Variables analyzed

At 30 and 60 DAS the height of the plants was measured with a graduated ruler; At 45 DAS the number of leaves and of inflorescence per plant were determined. After 60 days, the aerial parts of the plants were collected, washed with distilled water, conditioned in paper sacks and dried in forced air stove at 65°C during 48 hours. After drying, the weight of the dry biomass of the samples was determined.

Statistical analysis

The experimental data were analyzed by ANOVA. The data were subjected to analysis of variance using F test at 5% significance level for all analyzes. In case of significant

effect, it was proceeded regressions (linear and quadratic). All analyses were performed using statistical software Assisat 7.7

Conclusion

First results indicate that the nitrogen, phosphorus and potassium doses favored the growth of chia plants in relation to control, promoting an increase in all studied variables, evidencing the necessity of cultivation by these elements.

In general, considering the values of the variables according to the increasing doses of nutrients, a preliminary recommendation could be indicated, under the conditions of this research, as 125: 100: 40 kg ha⁻¹ of N: P: K. So the effect of these nutrients on chia need to be more investigated.

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