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Original article

Calcium and magnesium nanoparticle incidence on lemon productivity

Incidencia de las nanopartículas de calcio y magnesio sobre la productividad del limón Eureka

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ABSTRACT : Calcium and magnesium are essential elements in plant nutrition and there are currently fertilizers on the market made up of dolomite nanoparticles, which allow them to be incorporated into the soil or by foliar application. The objective of this work was to evaluate the incidence of calcium/magnesium nanofertilization on productivity, foliar nutrition and fruit quality of Eureka lemon. The following treatments were evaluated: 1: Control; 2: Nanoparticles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [foliar route]; 3: Nanoparticles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [foliar route]; 4: Nanoparticles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [applied to the soil]; 5 Nanoparticles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [applied to the soil]. It is in a Randomized Complete Block design with four replicates and four plants per replicate. Leaf samples were taken in March in two consecutive campaigns, determining concentrations of nitrogen, phosphorus, potassium, calcium, magnesium. At harvest, 40 fruits per experimental plot were selected, evaluating equatorial diameter, juice percentage, and production in kilograms per plant, content of total soluble solids, titratable acidity and maturity index. Calcium/magnesium nanofertilization applied via foliar 2 L ha^{-1} and applied to soil 4 L ha^{-1} were associated with foliar magnesium concentration, favoring an accumulation of this nutrient over time. The treatment with calcium/magnesium nanofertilizer in doses of 4 L ha^{-1} applied to the soil was significant in the percentage of fruit juice of 'Eureka' lemon plants.

Key words: *Citrus limon*, foliar nutrition, nanomaterials.

RESUMEN: El calcio y magnesio son elementos esenciales en la nutrición vegetal y actualmente en el mercado existen fertilizantes constituidos por nanopartículas de Dolomita, que permiten incorporarlos al suelo o vía foliar. El objetivo de este trabajo fue evaluar la incidencia de la nanofertilización de calcio/magnesio sobre la productividad, nutrición foliar y calidad de fruta del limón Eureka. Se evaluaron los siguientes cinco tratamientos: 1- Testigo; 2- nanopartículas Ca (9,8 %)-Mg (5,9 %) 2 L ha^{-1} [vía foliar]; 3- nanopartículas Ca (9,8 %)-Mg (5,9 %) 4 L ha^{-1} [vía foliar]; 4- nanopartículas Ca (9,8 %)-Mg (5,9 %) 2 L ha^{-1} [aplicado al suelo]; 5- nanopartículas Ca (9,8 %)-Mg (5,9 %) 4 L ha^{-1} [aplicado al suelo], distribuidos en un diseño de bloques completos al azar con cuatro repeticiones y cuatro plantas por repetición. Se tomaron muestras foliares en marzo, en dos campañas consecutivas, determinándose concentraciones de nitrógeno, fósforo, potasio, calcio, magnesio. A la cosecha se seleccionaron 40 frutos por parcela experimental, evaluándose diámetro ecuatorial, porcentaje de jugo, producción en kilogramos por planta, contenido de sólidos solubles totales, acidez titulable e índice de madurez. La nanofertilización de calcio/magnesio aplicado vía foliar 2 L ha^{-1} y aplicado al suelo 4 L ha^{-1} presentó asociación con la concentración de magnesio foliar, favoreciendo una acumulación de este nutriente en el tiempo. El tratamiento con nanofertilizante de calcio/magnesio en dosis de 4 L ha^{-1} aplicado al suelo fue significativo en el porcentaje de jugo del fruto de plantas de limón 'Eureka'.

Palabras clave: *Citrus limon*, nutrición foliar, nanomateriales.

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INTRODUCTION

The use of nanotechnology in agriculture favorably affects crop nutrition and protection. Calcium and magnesium are essential elements in plant nutrition (1) and they are being incorporated into the fertilizer market in high purity Dolomite nanoparticles.

Calcium (Ca) forms an important part of the cell membrane constitution and accumulates between the cell wall and the middle lamella, where it interacts with pectic acid to form calcium pectate, which confers stability and maintains cell integrity (1-3). This nutrient acts as a cementing agent of cells, is closely related to meristematic activity, influences the regulation of enzymatic systems, the activity of phytohormones and increases tissue resistance to pathogens, increasing postharvest shelf life and nutritional quality (4). Symptoms of deficiency appear in leaves that have not reached their final size (stage 1:15 according to BBCH scale (5), plants in general lose vigor and fruits show splitting of the rind or splitting (1).

A constant supply of Ca absorbed by the root and transferred to the fruit is crucial for healthy fruit development. Long-distance transport of Ca is via xylem/apoplast pathways from the root to the upper parts, and in the case of fruit Ca uptake, fruit expansion is also a determinant for sap inflow that delivers Ca to the fruit (2,3,5).

Magnesium (Mg) in plant cells plays a specific role as an activator of enzymes involved in respiration, photosynthesis, and DNA and RNA synthesis. It is also part of the chlorophyll molecule. Mg is attributed to participate in fruit development, contributing to the work of fructose 1.6 diphosphatase, which regulates starch synthesis, a factor that can be a determining factor in the level of sugars and fruit quality. The lack of this mineral element is manifested by a yellowing of the leaf, which does not reach the entire surface, leaving a green filled V, with its apex pointing towards the apex of the leaf. Given the mobility of this element in the plant, the affected leaves are the oldest ones (1).

Nanomaterials or nanoparticles (NPs), are materials of very small dimensions (less than 100 nanometers) with an order of magnitude of 10-9 m. This group of materials includes several NPs (Zn, Fe, Ca, Mg, Cu, Ag, etc.), many of which have great potential for sustainable agriculture and reducing environmental impact (5,6).

Nanofertilizers address the issue of agriculture as a solution regarding the absorption of macro and micronutrients in plants, thus increasing agricultural production; acting as growth promoters by correcting deficiencies of microelements (7-9). Nutrient deficiency decreases not only crop productivity, growth, development, yield and quality (10-12), but also affects human health through the consumption of nutrient-deficient foods (13-16). In this sense, the use of nanoformulated micronutrients for the slow or controlled release of nutrients would stimulate the absorption process by plants, promote crop growth and productivity, and also contribute to maintaining soil health (5, 17,18).

In a greenhouse study, they explored the effect of various doses of potassium sulfate (K_2SO_4) nanoparticles (NPs) on alfalfa growth and physiological response under salt stress. A salt-tolerant genotype and a salt-sensitive genotype were selected based on germination under salt and planted in pots containing 2 kg of sand. The highest shoot dry weight, relative yield, root length, and root dry weight in both genotypes were obtained when using K_2SO_4NPsat the 1/8 level. The different doses of K_2SO_4NPssignificantly affected the Na/K ratio and the concentrations of Ca, P, Cu, Mn and Zn in plant tissue. Application of K_2SO_4NPsat a rate of 1/8 improved plant physiological response to salt stress by reducing electrolyte leakage, increasing catalase and proline content, and increasing antioxidant enzyme activity. These results suggest that the application of KNPs may have better efficiency than conventional K fertilizers in providing adequate plant nutrition and overcoming the negative effects of salt stress in alfalfa (19).

They compared the effects of nano-calcium carbonate and colloidal calcium carbonate and found that nano-calcium carbonate treatments were better at increasing calcium content when sprayed on Tankan(*Citrus tankan* Hayata) leaves (20).

Therefore, this work aims to evaluate the effect of foliar and soil application of a calcium/magnesium nanofertilizer on productivity, foliar macronutrient content and fruit quality of lemon plants (*Citrus limon* L.) variety 'Eureka'.

MATERIALS AND METHODS

The trial was carried out during the 2017-2018, 2018-2019 seasons in the locality of Santa Rosa, Corrientes, Argentina in a commercial lot Doña Sara establishment, georeferenced at 28°14'26.0 "S and 58°08'40.6 "W.

The experimental material was lemon plants (*Citrus limon* L.) var. 'Eureka' grafted on Rangpur lime (*C. limonia*), a combination commonly used in the region. It was worked in an eight-year old plot, in red yellow podzolic soil, with a density of 285-lemon plants ha^{-1}, in a planting frame of 7 meters between rows and 5 meters between plants.

The experimental design used was a randomized complete block design, with four replications and four plants per plot, the useful plants being the two central ones where the measurements were taken. The treatments evaluated are described in Table 1.

The products applied were conventional powdered Dolomite $CaMg(CO_3)_2$ and MIST-Ca/Mg® which is a source of high purity dolomite nanoparticles, and is presented in a floatable emulsion. It contains 9.80 % Ca and 5.90 % Mg.

In treatment 1 (Control), the addition of 2 kg Pt^{-1} of conventional dolomite was split in two moments, 50 % in December and 50 % in April of each year. All the plants in the trial were fertilized with a compound fertilizer 15-6-15-6, providing 15 % (N)-6 % (P_2O_5)-15 % (K_2O)-6 % (MgO),

Table 1. Treatments, form of application, doses (2 and 4 liters per hectare and per year both to the soil and foliar) and times of application (December, April and March)

Treatments	Application form	Annual dose	Application time
1	Soil	2 Kg de $CaMg(CO_3)_2$ Pt^{-1}	50 % December and 50 % April
2	Foliar	2 L ha^{-1}	50 % December and 50 % April
3	Foliar	4 L ha^{-1}	50 % December and 50 % April
4	Soil	2 L ha^{-1}	50 % December and 50 % April
5	Soil	4 L ha^{-1}	50 % December and 50 % April

Treatments 2, 3, 4, 5 and 6 have MIST-Ca/Mg® application

applied at a rate of 2 kg per plant (50 % in September and 50 % in March of each year).

Variables analyzed

In order to evaluate the nutritional status of the plants, leaf samples were taken at 7 months of age from fruiting branches, coming from spring sprouting, in March in each of the plants evaluated for each treatment in two consecutive seasons. The leaves were dried in an oven at 60 - 65 °C until constant weight, ground in a 20-mesh Willey type grinder. Nitrogen (N) concentrations were determined by the Kjeldhal method; phosphorus (P) by the Murphy-Riley method, potassium (K), calcium (Ca) and magnesium (Mg) by atomic absorption spectrometry (22).

Yields, measured in kilograms of fruit per plant, were taken on the total number of useful plants in each plot.

At harvest time, 40 fruits were taken at random per experimental plot to determine fruit quality traits, in which the following variables were determined: equatorial diameter (ED) in millimeters by digital caliper, juice percentage (PJ) = juice mass/fruit weight x 100, production in kilograms per plant (kg $plant^{-1}$) (P), total soluble solids content (TSS), titratable acidity (A), and maturity index (MI) = TSS/A.

Precipitation in mm of rainfall during the experimental period is presented in Table 2.

Statistical analysis

To evaluate differences between treatments, analysis of variance (ANOVA) was performed for the corresponding design, incorporating the year effect as a factor in the statistical model and Duncan's test ($\alpha \leq 0.05$). Prior to ANOVA, the data were subjected to normality tests, with the modified Shapiro-Wilks statistic ($\alpha \leq 0.05$).

A principal component analysis (PCA) was then performed to evaluate the effect of the treatment on all the variables evaluated. Biplot plots were made with the principal components in order to interpret and identify associations between observations (treatments) and variables in the same space. All analyses were performed using Infostat software (21).

RESULTS AND DISCUSSION

In general, in the first year of study, foliar N levels were between deficient values (<2.30 %) and without significant differences, P between low levels (0.10-0.12 %) in treatments 2, 3 and 5, with optimum levels in treatment 4 (0.13-0.15 %) and significantly different from the rest of the treatments with high values (0.17-0.20 %) in the control treatment. K contents were optimal for all treatments (0.71-1 %), except for treatment 2, which presented significantly low K values (0.50-0.70 %). Ca was between optimum values (3-5 %) in all treatments and Mg between optimum (0.25-0.45 %) and high (0.46-0.90 %) ranges, being treatment 3 the one that presented significantly higher levels of Mg with respect to the control (Figure 1).

In the second year of study, N contents were low (2.3-2.5 %) in treatments 3 and 4, optimal (2.51-2.80 %) in treatment 2 and in excess (>3 %) in treatments 1 and 5. Only significant difference is seen between treatment 4 and 5. P levels were found between low (0.10-0.12 %) to optimum (0.13-0.16 %), K between optimum (0.71-1 %) to high (>1.30 %) and Ca at high levels (5.10-6.50 %) with no significant differences between treatments, while Mg

Table 2. Amount of precipitation recorded in millimeters (mm) per season and per month

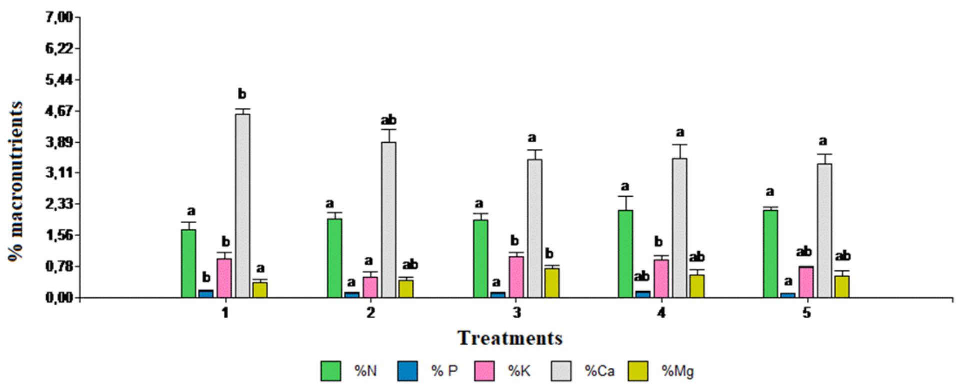
Month	Campaign 2017-2018	Campaign 2018-2019
August	68.0	68.0
September	96.0	91.0
October	93.0	53.0
November	96.0	450.0
December	56.0	350.0
January	200.0	336.0
February	90.0	76.0
March	172.0	171.0
April	80.0	228
May	50.0	165

presented high values (0.46-9 %) differentiating treatment 1 from 2 and 5 (Figure 2).

The association between treatment 2 [Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [foliar application]] and 5 [Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [applied to the soil]] and the Mg content in the plant was confirmed by corroborating that a significant increase in this nutrient was observed in these treatments (Figure 4).

Figure 3 shows the graphical representation of the Principal Component Analysis (PCA) of the fruit quality variables. The principal components PC1 and PC2 are observed, which explain 87.2% of the total variability. Treatments 3 and 4, with 4 L ha^{-1} of Ca and Mg nanoparticles applied by foliar application, and with Ca and Mg nanoparticles in doses of 2 L ha-^{1} applied to the soil, respectively, showed a greater association with the yield, equatorial diameter and fruit acidity variables; the control was associated with the variables TSS and MI; treatment 5, with Ca and Mg nanoparticles at a dose of 4 L ha^{-1} applied to the soil, was associated with the percentage of juice; while treatment 2, with Ca and Mg nanoparticles 2 L ha^{-1} via foliar, was not associated with any variable. It should be noted that the variables production in kg plant^{-1} (P), total soluble solids (TSS) in Brix, titratable acidity in % (A) and maturity index (MI) did not differ significantly among treatments (Table 3).

The association between treatment 5 and juice percentage was confirmed by corroborating that in the treatment a significant increase in this variable was observed in the samples evaluated, in spite of presenting statistically lower values of fruit equatorial diameter. Treatment 5 also presented association with high levels of foliar Mg, nutrient that is attributed participation in the development of fruits, contributing to the work of fructose



Treatments: 1 Control; 2 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha-^{1} [via foliar]; 3 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [via foliar]; 4 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [soil-applied]; 5 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [soil-applied]. *Different letters indicate significant differences (p≤0.05). Duncan's test (p≤0.05)

Figure 1. Foliar concentrations of macronutrients and standard error as a function of fertilization treatments in Lemon 'Eureka' trees, in Santa Rosa, Corrientes, Argentina, in the first year of study

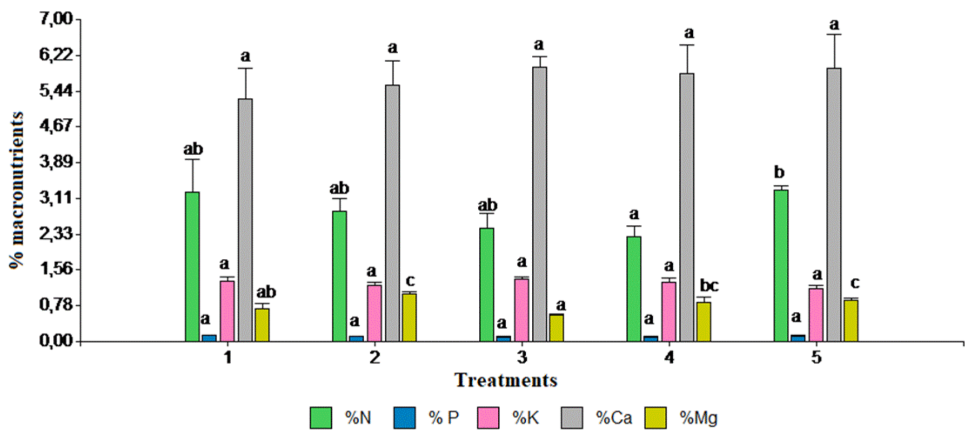


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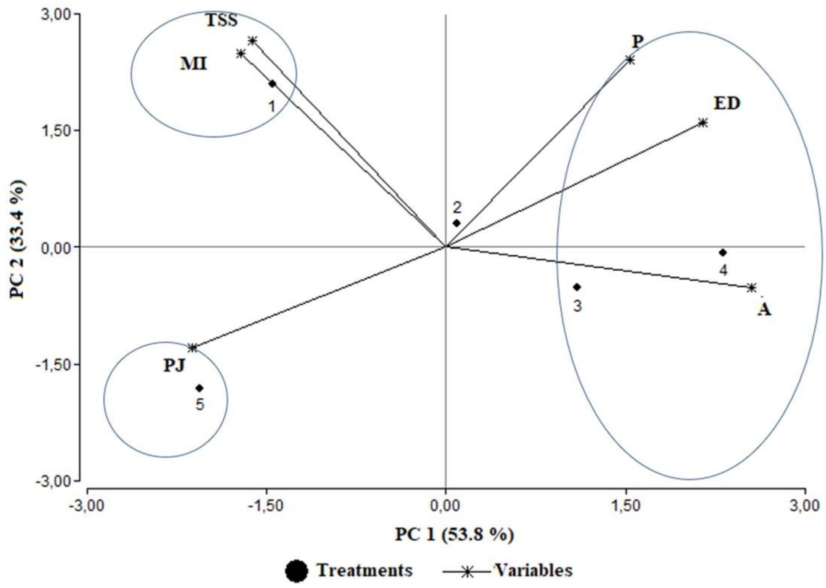
Figure 2. Foliar macronutrient concentrations and standard error as a function of fertilization treatments in Lemon 'Eureka' trees, in Santa Rosa, Corrientes, Argentina, in the second year of study



Equatorial diameter (ED), juice percentage (PJ), yield in kilograms per plant (kg plant^{-1}) (P), soluble solids content (SS), acidity (A) and maturity index (MI) in 'Euteka' lemon fruit for the five treatments tested in the seasons evaluated

Figure 3. Biplot resulting from the Principal Component Analysis (PCA) of the following variables

Table 3. Mean values ± standard error, by treatment of the quality variables: Production in Kg plant^{-1} (P), equatorial diameter (ED) in millimeters (mm), percentage of juice (PJ), total soluble solids (TSS) in Brix degrees, titratable acidity in % (A) and maturity index (MI)

Treatment	P	ED	PJ	TSS	A	MI
1	1405.75a	65.80 b	41.85ab	7.55ª	5.60a	1.35a
2	1320.83a	63.60ab	39.45ab	7.55ª	5.75a	1.32a
3	1394.00a	64.73 b	41.90ab	7.50ª	5.78a	1.30a
4	1398.00a	66.10 b	36.56a	7.35ª	5.82a	1.26a
5	1225.53a	**60a**	**43.42 b**	7.50ª	5.63a	1.33a
EE	55.95	1.47	1.92	0.18	0.12	0.04

Treatments: 1 Control; 2 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [via foliar]; 3 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [via foliar]; 4 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [soil applied]; 5 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [soil applied]. *Different letters indicate significant differences (p≤0.05). Duncan's test (p≤0.05)

1,6 diphosphatase, which regulates the synthesis of starch, factor that can be determinant in the level of sugars and the quality of fruits (1).

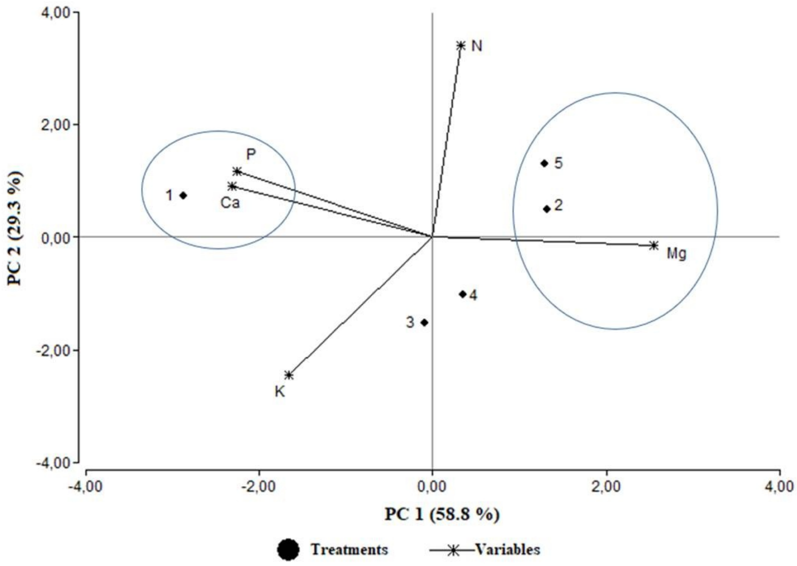
The constant supply of nanoparticulate Ca and Mg, which are absorbed by the root in soil applications and by stomatal openings in foliar applications, can then be transported to various sites of the plant through the xylem and phloem routes (5). A fact that is corroborated by the results of the second year of the study, where high concentrations of these elements were found in all treatments.

Long distance transport of Ca is through xylem/apoplast pathways from the root to the upper parts, and on the fruit side, fruit expansion is also a determinant for sap inflow that delivers Ca to the fruit (2,3), which is crucial for healthy fruit development.

Studies indicate that Ca content and fruit size were the highest in *Citrus reticulate* cv. Shatangju and cv. Mashuiju, and the lowest values in grapes (*Vitis vinifera* cv. Summer black and cv. Shine Muscat. Regarding the Ca nutritional value of table fruits, the Ca concentration in the pulp is more important than the Ca content in the whole fruit, which contains inedible tissues such as stones and leathery skin. Loquat and citrus fruits presented the highest Ca content in the flesh and they can be a good source of calcium nutrition (24).

The Principal Component Analysis (PCA) and Biplot of the nutritional variables analyzed and the fertilization with dolomite nanoparticles explained 88.1 % of the association between the variables in two components. Principal Components 1 and 2 (PC 1, PC 2) accounted for 58.8 and 29.3 %, respectively, of the total variation (Figure 4).

Treatments 2 [Ca (9.8 %)-Mg (5.9 ha %) 2 L ha^{-1} [via foliar]] and 5 [Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [applied to the soil]], presented greater association with foliar magnesium concentration. It favors an accumulation of this nutrient over time, presenting optimum values (23), in the first year of study (0.43 and 0.45 % Mg respectively) to values in excess



Treatments: 1 Control; 2 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [foliar]; 3 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [foliar]; 4 Nano particles Ca (9.8 %)-Mg (5.9 %) 2 L ha^{-1} [soil applied]; 5 Nano particles Ca (9.8 %)-Mg (5.9 %) 4 L ha^{-1} [soil applied]

Figure 4. Biplot resulting from the Principal Component Analysis (PCA) of the nutritional variables (macronutrients) in Lemon 'Eureka'Eureka' in the five treatments tested

(>0.90 %) and high (0.46 - 0.90 %) in the second year (Figure 1 and 2). Treatment 1 (control) was associated with Ca and P content, while treatments 3 and 4 were not associated with no variable.

CONCLUSION

The contribution of calcium and magnesium nanofertilizer applied via foliar at a dose of 2 L ha^{-1} and applied to the soil at a dose of 4 L ha^{-1} showed a greater association with the concentration of foliar magnesium, favoring an accumulation of this nutrient over time. It is with optimal values in the first year of the study and high values in excess and high values in the second year. An association was also found between the treatment with Ca and Mg nanofertilizer at a dose of 4 L ha^{-1} applied to the soil and the percentage of fruit juice. The increase in foliar magnesium had an effect on at least one parameter of fruit quality in 'Eureka' lemon plants.

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