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# Evaluation of the application of fertilizers and biostimulants in *Zephyranthes lindleyana* Herb (Amarylidaceae) under greenhouse conditions

**Rodríguez-Flores, Zayner E.<sup>1</sup>; Fernández-Pavía, Yolanda L.<sup>1\*</sup>; García-Cué, José L.<sup>1</sup>; De la Cruz-Torres, Eulogio<sup>2</sup>; Jaen-Contreras, David<sup>1</sup>**

<sup>1</sup> Colegio de Postgraduados-Campus Montecillo, Carretera México-Texcoco km 36,5, Montecillo, Texcoco, Estado de México, C.P. 56264.

<sup>2</sup> Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca-La Marquesa, Ocoyoacac, Estado de México, C.P. 52750.

\* Correspondence: mapale1@colpos.mx

## ABSTRACT

**Objective:** To evaluate the effect of different doses of N, P, K fertilizers and two biostimulants, on growth parameters and vegetative development in plants of the species *Z. lindleyana* Herb.

**Design/methodology/approach:** An experiment was established under greenhouse conditions under a CRD; ten treatments with five repetitions were tested, with different doses of N, P and K and two biostimulants. The experimental unit was one plant per pot. Data were analyzed with descriptive statistics, non-parametric Kruskal-Wallis tests ( $\alpha=0.05$ ). Budding, flowering, leaf height, number of leaves and seed production were measured.

**Results:** Vegetative development was distinguished in all plants. Only six treatments showed flowering. The percentage of floral and vegetative sprouting did not show delay or advance in the physiology of the plant. In leaf height and number of leaves, different behaviors were detected in the treatments, not detected in the Kruskal-Wallis tests. The plants treated with high doses of NPK fertilization and combined with biostimulants showed inflorescences and seed production.

**Limitations on study/implications:** Being a native plant with ornamental potential, the collection of this species is a challenge due to the lack of studies on the potential distribution of the species.

**Findings/conclusions:** This species can be cultivated under a greenhouse and pot planting system. Floral budding, height and number of leaves is stimulated by high doses of nitrogen. The combination of NPK and biostimulants favors development/growth and seed production.

**Keywords:** biodiversity, biomass, endemic, nutrient, ornamental.



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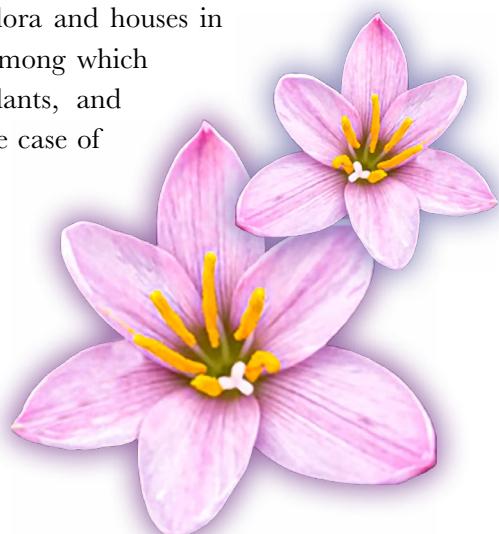
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## INTRODUCTION

Mexico occupies the fifth place in wealth of flora and houses in its territory between 25,000 and 30,000 plants, among which around 11,000 species are endemic vascular plants, and which offer a broad ornamental potential, as is the case of some genera such as *Zephyranthes*, *Hymenocallis* and *Sprekelia* whose species are not used for commercial production (Mapes and Basurto, 2016; Villaseñor, 2016).

The genus *Zephyranthes* contains approximately 90 species of which 37 are native to Mexico, which are distributed in subtropical and



tropical regions (Fischuck, 2021), primarily in the north and center-south of the country (Afroz *et al.*, 2018; Spurrier *et al.*, 2015). On the other hand, *Zephyranthes lindleyana* Herb. is a native species from the Mexican territory with high ornamental potential (Leszczyńska *et al.*, 2000), and the same as other species such as *Z. citrina*, *Z. carinata* and *Z. minuta* lack reliable reports in different areas, from taxonomic to agronomic management, since there is scarce information published about the optimal doses of fertilization. Karavidas *et al.* (2022) point out that, as the biological basis for primary production, practices such as fertilization in endemic plants must be established, since these practices allow preserving and potentiating natural resources.

The study of endemic plants promotes a scenario so that those that are native are sources of biological and genetic resources that promote the diversity of crops and at the same time allow their conservation. The potential of these native species, such as *Zephyranthes lindleyana* Herb., as a result of their value as ornamental and phytochemical product, creates the opportunity to contribute to the increase of genetic diversity and allows new opportunities for species with ornamental potential to be traded. Cardoso and Vendrame (2022) consider that in order to achieve this, it is necessary to identify the ideal nutrition that allows conserving genetic resources since there is the danger of losing these species due to climate change or the disturbance of their habitats.

Because of the aforementioned, the following question emerged: What effect does the application of different doses of N, P and K and biostimulants have on *Z. lindleyana* Herb (Amarylidaceae)? To answer it, a study was conducted that had the objective of evaluating the effect of different doses of N, P, K fertilizers and two biostimulants, on growth parameters and vegetative development in plants of the species *Z. lindleyana* Herb (Amarylidaceae). The hypothesis proposed was that different doses of fertilization and two biostimulants have an influence on the growth and development of the plant. There is the intention to induce the ideal development and growth of *Z. lindleyana* Herb., with the addition of different doses of N, P and K and two commercial biostimulants based on brown algae (*Ascophyllum nodosum*), under homogeneity of the substrate, planting methods, irrigation, and greenhouse conditions to make known the management of this endemic species.

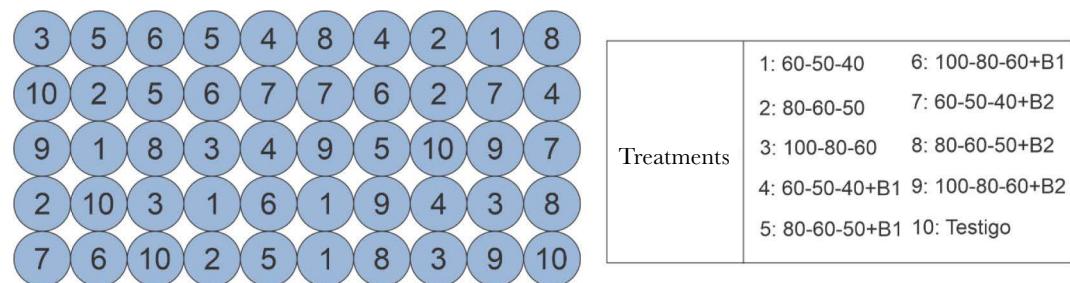
## MATERIALS AND METHODS

### Location and experimental conditions

The experiment was conducted within the facilities of the Research Unit “Ramón Fernández González” in the urban locality of San Miguel Coatlinchán, Texcoco, Estado de México (19° 27' 47.19" N and 98° 52' 54.71" W and 2272 masl) with coordinates. The experimental site has a semi-dry temperate climate with maximum annual temperature of 25 °C and minimum of 7.7 °C, as well as a maximum seasonal rainfall of 3.8 mm in the summer and a minimum of 0.3 mm in the winter. The plant material was donated by the Centro de Investigación en Plantas Nativas of the Universidad Popular Autónoma del Estado de Puebla, Atlixco, Puebla, Mexico, collected in the limits of the municipality of Tepeojuma e Izúcar de Matamoros, Puebla, Mexico.

### Treatments and experimental design

An experiment was established with plants from the species *Z. lindleyana* Herb. under greenhouse conditions established with a completely randomized design, where ten treatments were tested with five repetitions (Figure 1). The treatments were designed according to the Exploratory Batch Technique (FAO, 2022). The experimental unit was one plant per pot.



**Figure 1.** Distribution plan of the treatments of the fertilization experiment. Doses of N, P, K expressed in percentage. b1: Biostimulant MC Cream®; b2: Biostimulant MC Extra®.

### Management of the experiment

Planting was carried out in the month of March 2021 in black polyethylene bags with capacity of 3 L. The physicochemical properties of the substrate were determined before sowing (Table 1), and the texture was reported as Sandy Loam (SL). The irrigation management was manual every third day using tap water. One week after sowing, when vegetative sprouting happened in most of the plants, application of the treatments was conducted.

**Table 1.** Physicochemical characterization of the soil.

pH	E.C dS m <sup>-1</sup>	O.M. %	N %	P mg kg <sup>-1</sup>	CEC meq/100 g	Fe	Cu	Zn	Mn
						mg kg <sup>-1</sup>			
5.4	2.2	12.4	0.6	23	32	65	2	39	80

E.C.: Electric Conductivity. O.M.: Organic Matter, CEC: Cation Exchange Capacity.

### Data analysis

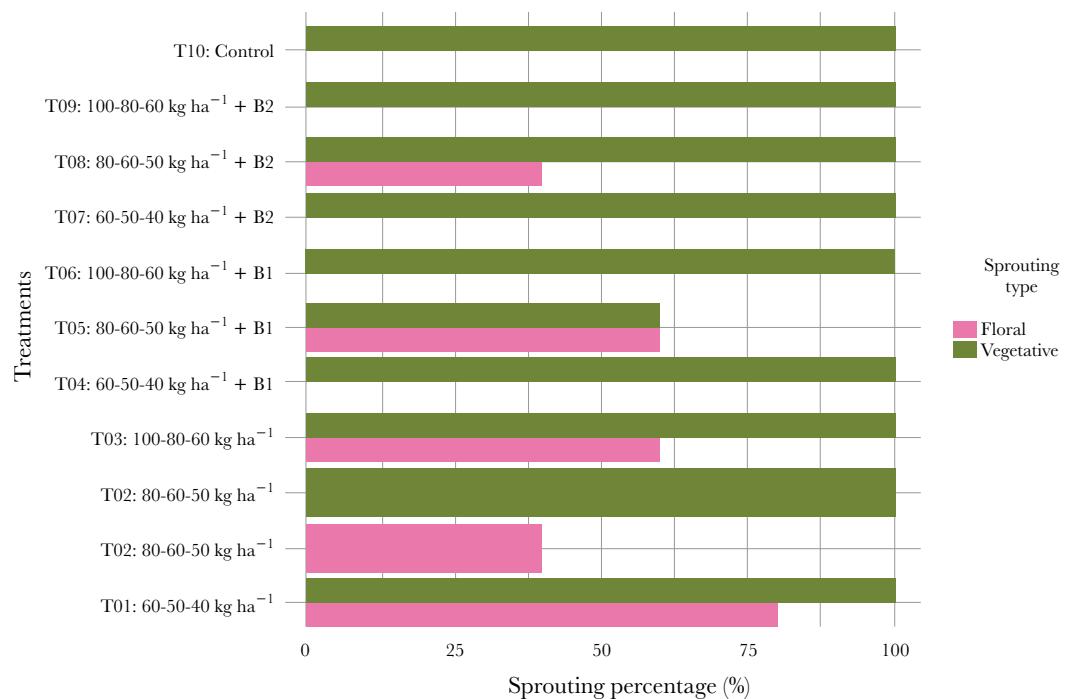
Data analysis was done in different ways. For the sprouting and flowering, the percentage of floral and vegetative sprouting was calculated per treatment. These measurements were conducted in a range of three days since the first sprouting and flowering. To quantify the vegetative development, measurements were carried out in botanical terms of plant height or leaf length (LH) and number of leaves (NL). Taking these measurements was done periodically with a millimetric ruler of 30 cm. The height was taken from the neck of the bulb and start of the leaf to the apex of the largest leaf. The growth response of the plants was provided by taking data in the flowering stage, which is when it expresses its greatest growth habit and expression of the plant, according to the crop's physiology. Then, the LH and NL data were analyzed with univariate descriptive statistics. Furthermore, the

normality of the variables was tested through the Shapiro Wilk test ( $\alpha=0.05$ ). Next, the nature of the variables and the results were analyzed. Since the assumption of normality was not fulfilled, the decision was made to use non-parametric Kruskal-Wallis tests ( $\alpha=0.05$ ) to identify similarities or differences between their medians. Seed production was calculated through the total number per treatment and then the treatments were compared by their median with the Kruskal-Wallis test ( $\alpha=0.05$ ).

## RESULTS AND DISCUSSION

During the month of April, vegetative sprouting began in most of the treatments in a uniform manner, except for the treatments T05 ( $80-60-50 \text{ kg ha}^{-1} + \text{MC Cream}^{\circledR} 1\text{mL L}^{-1}$ ) and T09 ( $100-80-60 \text{ kg ha}^{-1} + \text{MC Extra}^{\circledR} 0.5\text{g/L}$ ) which only obtained 60% of sprouting. The floral emergence began during the second week of May 2021.

The dose  $80-60-50 \text{ kg ha}^{-1}$  was also where these buds were most present in T02, T05 and T08 (Figure 2). The dates agree with what was reported by Tapia *et al.* (2017) which coincide with the genus Z. The presence of floral buds was found in treatments T01, T02 and T03 which do not have biostimulants. The plants presented different behavior in the different treatments that were noted visually in a qualitative manner.



**Figure 2.** Percentage of floral and vegetative sprouting per treatment.

The univariate descriptive analyses on LH (Table 2) evidence the behavior by treatment. In the minimum values, it can be observed that the treatments T04 and T09 obtained values of 1 cm height, while the treatments T01, T07 and T08 exceeded 10 cm. In maximum values, this difference is evidenced in the same way since all the plants

**Table 2.** Descriptive statistics of the variable leaf height (LH) of *Zephyranthes lindleyana* Herb. plants under different nutrition treatments.

Trat	Leaf height (cm)						
	Min	Max	Mean	Median	Std.dev	Coef. var	Pl
T01	10.4	19.5	16.36	17.5	3.8	23.22	5
T02	5	24.6	18.78	21.4	7.8	42.01	5
T03	4.9	27	16.6	14	9.61	57.73	5
T04	1	24.7	15.78	17.9	9.09	57.61	5
T05	6.7	24.6	17.22	19.2	7.51	43.64	5
T06	2	25	17.7	22.5	9.73	55.01	5
T07	14.8	25	21.66	22.9	3.97	18.33	5
T08	14.2	21	17.72	17.9	2.42	13.65	5
T09	1	24.3	12.78	10.4	9.95	77.86	5
T10	4.3	20	14.24	13.5	6.45	45.29	5

Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean: Mean, Median: Median, Std.var: Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha<sup>-1</sup>, T02: 80-60-50 kg ha<sup>-1</sup>, T03: 100-80-60 kg ha<sup>-1</sup>, T04: 60-50-40 kg ha<sup>-1</sup> + MC Cream, T05: 80-60-50 kg ha<sup>-1</sup> + MC Cream, T06: 100-80-60 kg ha<sup>-1</sup> + MC Cream, T07: 60-50-40 kg ha<sup>-1</sup> + MC Extra, T08: 80-60-50 kg ha<sup>-1</sup> + MC Extra, T09: 100-80-60 kg ha<sup>-1</sup> + MC Extra, T10: Control.

surpassed 20 cm of height, the effect of eight of nine treatments exceeded the control T10, of which the tallest heights corresponded to treatments with high doses of nitrogen with and without biostimulants (T02:80-60-50 kg.ha<sup>-1</sup> and T06:100-80-60 kg.ha<sup>-1</sup>+MC Cream® 1mL/L). Treatment T07 (60-50-40 kg.ha<sup>-1</sup>+MC Extra®) exceeded the control by nearly 10 cm. The coefficients of variation in T01, T07 and T08 are low, and in the others there is a heterogeneous behavior within the treatment. Then, a non-parametric Kruskal-Wallis test was carried out, where no differences were distinguished between the medians of the treatments ( $\chi^2=6.6324$ , P value=0.6753).

For NL the same procedure was followed and after performing the descriptive statistics (Table 3), the results were also contrasting, since the vegetative development was not homogenous in the treatments. The minimum values show that leaf formation for some treatments was affected importantly, since some treatments only obtained one leaf in a plant; however, since it was a discreet variable, the best value to represent a difference, descriptively, is the median. The value in which the best treatments were found quantitatively were treatments T02 (80-60-50 kg ha<sup>-1</sup>) and T06 (100-80-60 kg.ha<sup>-1</sup>+MC Cream® 1mL/L) with a median of six leaves. The lowest value was the control T10. The coefficients of variation in T01, T07 and T08 are low, and in the others, there is a heterogeneous behavior within the treatment. Likewise, a non-parametric Kruskal-Wallis test was carried out, where no differences were found between the treatment medians ( $\chi^2=8.6305$ , P value=0.4721).

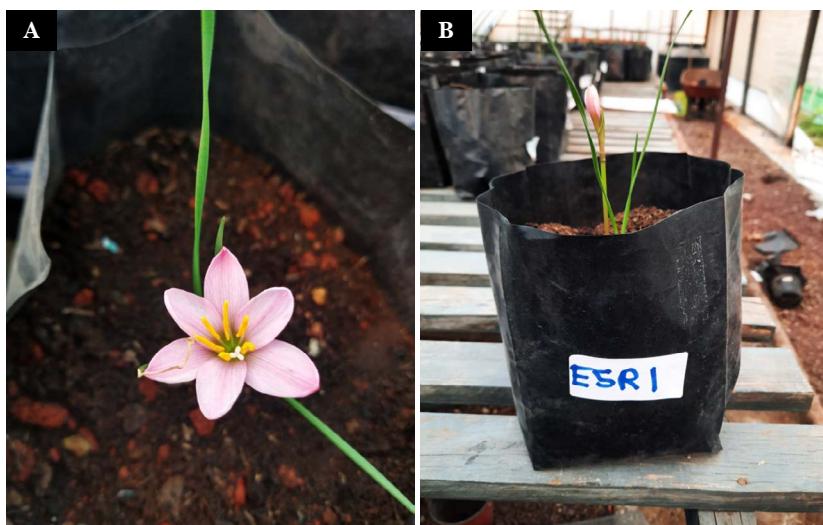
When contrasting the results obtained with others reported in species of the same family (such as *Iseme amancaes*), subjecting the plants to different doses of NPK did not show a significant effect in growth variables (Jaulis & Pacheco, 2018); however, there are

**Table 3.** Descriptive statistics of the variable number of leaves (NL) of *Zephyranthes lindleyana* Herb. plants under different nutrition treatments.

Trat	Number of leaves						
	Min	Max	Mean	Median	Std.dev	Coef.var	Pl
T01	3	5	3.8	4	0.83666	22.01	5
T02	1	8	5.2	6	2.5884358	49.77	5
T03	2	5	3.8	4	1.3038405	34.31	5
T04	1	6	4	4	1.8708287	46.77	5
T05	1	6	3.8	4	1.9235384	50.61	5
T06	2	8	5.2	6	2.280351	43.85	5
T07	3	6	4.8	5	1.0954451	22.82	5
T08	4	6	5	5	0.7071068	14.14	5
T09	1	6	3.6	4	2.0736441	57.60	5
T10	1	5	6.4	3	1.6733201	49.21	5

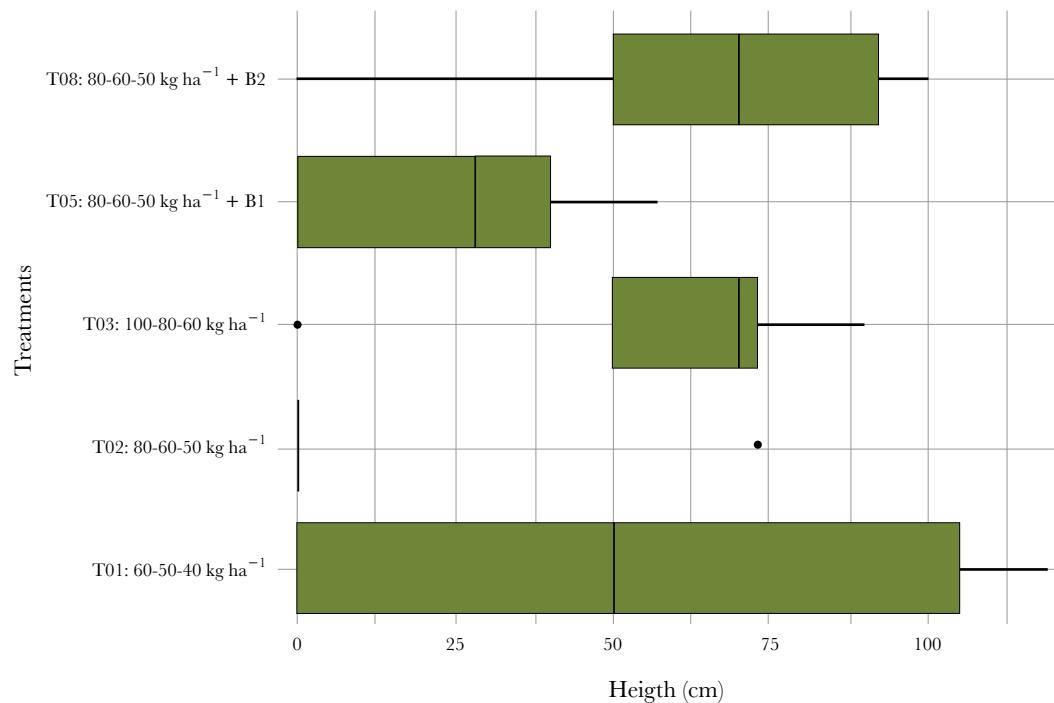
Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean: Mean, Median: Median, Std.var: Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha<sup>-1</sup>, T02: 80-60-50 kg ha<sup>-1</sup>, T03: 100-80-60 kg ha<sup>-1</sup>, T04: 60-50-40 kg ha<sup>-1</sup> + MC Cream, T05: 80-60-50 kg ha<sup>-1</sup> + MC Cream, T06: 100-80-60 kg ha<sup>-1</sup> + MC Cream, T07: 60-50-40 kg ha<sup>-1</sup> + MC Extra, T08: 80-60-50 kg ha<sup>-1</sup> + MC Extra, T09: 100-80-60 kg ha<sup>-1</sup> + MC Extra, T10: Control.

other studies that report that the combination of high doses of nitrogen and biostimulants show improvements in other parts of the plant such as the root, thickness, length, weight and number of bulbs (Anbarasi & Haripriya, 2020). During the first week of the month of May, T01 (60-50-40 kg ha<sup>-1</sup>), T03 (100-80-60 kg ha<sup>-1</sup>), T05 (80-60-50 kg ha<sup>-1</sup> + MC Cream<sup>®</sup> 1mL L<sup>-1</sup>) and T08 (80-60-50 kg ha<sup>-1</sup> + MC Extra<sup>®</sup> 0.5g/L) were the only ones that presented inflorescences. The inflorescences behaved in the same way in all the treatments; in each of the repetitions, only a single magenta flower was produced (Figure 3).



**Figure 3.** Floral expression and morphology of the *Zephyranthes lindleyana* Herb. plant. A: Solitary flower with magenta color, B: Complete plant of *Z. lindleyana* Herb.

One of the flowering variables was the stem height (SH), measurement indicating the flower stem height. The number of flowers in all the treatments was from a single solitary flower with the same characteristics. Figure 3 shows the treatments that did obtain flower. It can be seen that there is a wide dispersion in the treatments because not all plants presented inflorescences. Likewise, it can be seen that treatment T08 (80-60-50 kg ha<sup>-1</sup> + MC Extra<sup>®</sup>) was the one that behaved in the least dispersed way and with a mean of around 7 cm of height, which contrasts with treatment T05 (80-60-50 kg ha<sup>-1</sup> + MC Extra<sup>®</sup>). However, in some treatments, as in T01 where all the repetitions presented inflorescences, not all the flowers produced seeds because the fruit did not reach maturity or simply was detached before producing them (Figure 4).



**Figure 4.** Stem height of *Zephyranthes lindleyana* Herb. from the treatments that presented inflorescence.

It should be highlighted that in the treatments where a biostimulant was not included, there was seed production, although the dose that did produce seeds with or without biostimulants was the 80-60-50 kg ha<sup>-1</sup> dose, even if this production was affected in its application without biostimulant (T02). A Kruskal-Wallis test was conducted between treatments T01, T02, T03, T05 and T08 and no statistically significant differences were identified for the values of the average number of seeds. Table 4 shows that not all the plants of the corresponding treatments produced seeds, and in fact only two plants could produce seeds in treatments T02 and T08. Likewise, when comparing the maximum number of each treatment (except T02) the seed production exceeded 30 units. Taking into account the number of plants and the mean, it can be defined that qualitatively the treatment with highest number of seeds corresponded to the lowest doses of N, P, K without biostimulant T01 (60-50-40 kg ha<sup>-1</sup>).

Table 4 shows the descriptive statistics of the number of plants sprouted that gave seeds. It was detected that in T02 and T08 only two plants could produce them from a total of five. Treatment T01 was the one with highest production with a total of 101 seeds that corresponded to the lowest dose of N, P, K without biostimulant (T01: 60-50-40 kg ha<sup>-1</sup>). The treatments (T01, T02 and T03), where no biostimulant were included, had some plants with seed production. The dose 80-60-50 kg ha<sup>-1</sup>s produced seeds with and without biostimulants. This dose produced less seeds in treatment T02 where no biostimulant was applied. When applying an unbalanced Kruskal-Wallis non-parametric test (by the number of plants where seeds emerged), the following was obtained:  $\chi^2=4.41$ , P value=0.3531; that is, no statistical differences were found in the medians of the seeds per treatment (P≤0.05).

**Table 4.** Descriptive statistics of the number of plants emerged that gave seeds of *Zephyranthes lindleyana* Herb.

Trat	Seeds (number)								
	Min	Max	Mean	Median	Std.dev	Coef.var	SE. mean	Suma	Pl
T01	20	34	25.25	23.50	6.7019	26.54	3.350	101	4
T02	15	16	15.50	15.50	0.7071	4.56	0.500	31	2
T03	11	42	27.00	28.00	15.524	57.49	8.962	81	3
T05	15	42	26.66	23.00	13.868	52.00	8.006	80	3
T08	30	53	41.50	41.50	16.263	39.18	11.50	83	2

Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean: Mean, Median: Median, Std.var, Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha<sup>-1</sup>, T02: 80-60-50 kg ha<sup>-1</sup>, T03: 100-80-60 kg ha<sup>-1</sup>, T04: 60-50-40 kg ha<sup>-1</sup> + MC Cream, T05: 80-60-50 kg ha<sup>-1</sup> + MC Cream, T06: 100-80-60 kg ha<sup>-1</sup> + MC Cream, T07: 60-50-40 kg ha<sup>-1</sup> + MC Extra, T08: 80-60-50 kg ha<sup>-1</sup> + MC Extra, T09: 100-80-60 kg ha<sup>-1</sup> + MC Extra, T10: Control.

## CONCLUSIONS

The hypothesis proposed is not rejected. The native species *Zephyranthes lindleyana* Herb (Amarylidaceae) responded to the application of different doses of NPK and biostimulants in a sandy loam soil. *Z. lindleyana* Herb. can be cultivated in the central zone of the country under a greenhouse planting system and plot without showing an advance or delay in vegetative and floral sprouting. Floral emergence will be stimulated by high doses of nitrogen and show different responses in variables of height and number of leaves. The effect in treatments with different doses of NPK and biostimulants favored the development or growth of plants and the production of seeds. This was noted with regards to the control treatment.

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