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RESEARCH ARTICLE

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Effect of synthetic fertilization dose on the diameter increase, height and mortality of *Cinchona officinalis* L. (Rubiaceae)

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ABSTRACT

Cinchona officinalis, is a South American tree species, commonly used for medicine, and is currently threatened by agricultural expansion and cattle ranching. The objective was to determine the effect of chemical fertilization on the nursery growth to increase growth potential and survival of *C. officinalis*. A completely randomized design with six treatments and three replicates was used; 20 *C. officinalis* plants were used per replicate. Two months after transplanting the *C. officinalis* seedlings to the polyethylene bags, inorganic fertilizer (YaraMila® HYDRAN) was applied. Monthly evaluations were carried out and the number of dead plants, plant height, diameter and number of leaves were recorded. The highest mortality rate was recorded when fertilizer was applied (73%) while in the non-fertilized treatment mortality reached 36%. Regarding the increase in height, diameter and number of leaves in all cases, the best results were obtained in the fertilized treatments, exceeding by 85, 70 and 17% (respectively) those obtained in the treatment to which fertilization was not applied. This study shows the effects that the application of fertilizers to *C. officinalis* plants at the nursery level can have on growth and mortality variables, the results suggest the use of this product for a sustainable and large-scale production of this species taking into consideration the appropriate dosage.

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Introduction

Cinchona officinalis is a forest species of medicinal importance, as it was the only cure for malaria for more than three centuries (Cóndor et al. 2009; Bharadwaj et al. 2018) and its alkaloids are considered to be the most influential tree bark-derived medicine in human history (Prendergast and Dolley 2001).

Cinchona officinalis needs specific conditions to ensure its growth, and the distribution range is limited (Armijos-González and Pérez-Ruiz 2016). The species faces different negative conditions that hinder the location of *C. officinalis* populations in natural forests (Buddenhagen et al. 2004). The first problem is regarding its natural habitat, since it has been affected by migratory farming and agriculture (Huamán et al. 2019; Fernandez et al. 2022). Second problem, is the low survival rate of seedlings in nursery (Fernandez et al. 2021) and finally the limited natural regeneration due to its low germination capacity influenced by factors such as seed quality, humidity, temperature and microbial activity (De-la-Cruz et al. 2007; Santos et al. 2010; Valdiviezo et al. 2018). These conditions have driven the prioritization of the conservation and recovery of *C. officinalis* in Peru (Albán-Castillo et al. 2020).

To ensure the success of afforestation and reforestation plans from the establishment of *C. officinalis* seedlings grown in forest nurseries, it is necessary to standardize protocols for obtaining seedlings suitable for the edaphoclimatic conditions of the area to be restored (Massone et al. 2018). Among the parameters to consider are management factors, fertilization (chemical or organic fertilization) is an activity that can significantly influence plant growth, health and productivity, as well as increase plant tolerance to many biotic and abiotic stresses (Souri and Hatamian 2019; Serri et al. 2021).

During the search for scientific information, no report was found in which the response of *C. officinalis* to the application of inorganic fertilizers had been studied, so this research is pioneering and

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generating a range of opportunities to continue developing research in this area, in addition, the results of this research will allow optimizing the management of fertilization of *C. officinalis* at the nursery level and ensure the quality of seedlings to be used in reforestation programs (Oliet et al. 2005). In this context, the study sought to determine the effect of the application of inorganic fertilizer application on some growth parameters and mortality of *C. officinalis* at the nursery level.

Materials and Methods

Study area

The research was carried out in the La Cascarilla locality (5° 40′ 21.12″ S and 78° 53′ 55.65″ W), province of Jaén, Peru, which is located at 1810 m. In this area the annual precipitation is 1730 mm, the minimum temperature is 13.0 °C and the maximum is 20.5 °C (Fernandez et al. 2021; Fernandez et al. 2022).

Plant material

C. officinalis seedlings were obtained from seeds. These were collected from a single population, from the locality of San Luis (6°22′ 6.68″ S and 79°3′ 29.50″ E) at 2489 m altitude. We collected 0.5 kg of mature capsules (brown to dark brown in color), packed in cloth bags and transported them to the community of La Cascarilla, where they were stored in the shade. After 20 days, seeds without visible cracks, fungi and/or nematodes were selected for use in the study (Fernandez-Zarate et al. 2022). For germination, an subirrigation chamber described by (Fernandez et al. 2021) was used, seeds were sown in forest substrate (Fernandez-Zarate et al. 2022). *C. officinalis* seedlings were transplanted into 269.25 cm^3 polyethylene cylindrical bags (7 cm diameter and 7 cm high).

Soil characterization

The physical and chemical properties of the substrate used for the transplanting of *C. officinalis* were determined before applying the fertilizer and at the end of the trial. The methods used were:

For texture: hydrometer method, for electrical conductivity (EC) was determined in aqueous extract soil: water (1:1), organic matter (OM): method established by Walkley and Black, nitrogen (N): Kjeldahl method, phosphorus (P): Olsen method, cation exchange capacity (CEC) and exchangeable cations: saturation with ammonium acetate, all analyses were determined at the Soil and Water Research Laboratory of the National University Toribio Rodriguez de Mendoza.

The initial characteristics of the substrate were: N = 0.34%, P = 14.9 ppm, magnesium, K = 398 ppm, (Mg) = 1.58 meq/100g, calcium (Ca) = 10.2 meq/100g, pH = 5.59, OM = 6.88%, CEC = 14.97 meq/100g, EC = 0.14 dS/m and textural class = sandy loam.

Experimental design

A completely randomized design with six treatments and three replicates per treatment was used; 20 *C. officinalis* plants per replicate and 360 plants were used throughout the trial. Two months after transplanting *C. officinalis* to the polyethylene bags, inorganic fertilizer (YaraMila® HYDRAN) was applied and then followed up monthly. This fertilizer is composed of NPK and micronutrients; it is also one of the most widely used fertilizers in Peru. The nutritional composition of which is shown in Table 1.

Measurements

To determine the effect of fertilization on nursery growth of *C. officinalis*, monthly evaluations were conducted for six months to record the number of dead plants, plant height (from the top of the polyethylene bag to the apex of the plants), diameter (measured with a digital vernier at substrate level).

Data analysis

Data were processed in StatGraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, VA, USA). The assumptions of normality (Shapiro Wilk) and homogeneity of variances (Levene's test) were checked. Then, an analysis of variance (ANOVA) was performed for each response variable and the mean values were compared through Tukey's test (α = <0.05), in addition, a correlation test was performed in the R programming environment between the growth variables evaluated and the chemical properties of the substrate at the end of the trial.

Results

Thirty days after applying the fertilizer to the *C. officinalis* plants, the first records of mortality were observed in treatments T4, T5 and T6, whose trend is sustained throughout the trial, reaching percentages of mortality of 64, 73 and 54% respectively, it can also be seen that treatment T2 reports its first mortality data 60 days after fertilizer application, reaching 73% mortality at the end of the trial (Figure 1A). Finally, the treatment to which no fertilizer was applied reported the lowest mortality rate (36%), which suggests that the application of chemical fertilizers on *C. officinalis* has a negative impact on plant survival at the nursery level.

It can be seen that in T5 there is a decrease in the number of leaves until day 90, thereafter a linear

Table 1. Fertilizer dose distribution according to trial treatment.

Treatment	Dose (g/planta)	Nutrients (%)
T1	0	N = 19, P_2O_5=4, K_2O = 19, MgO = 3
T2	0.3	
T3	0.5	
T4	0.7	
T5	0.9	
T6	1.1	

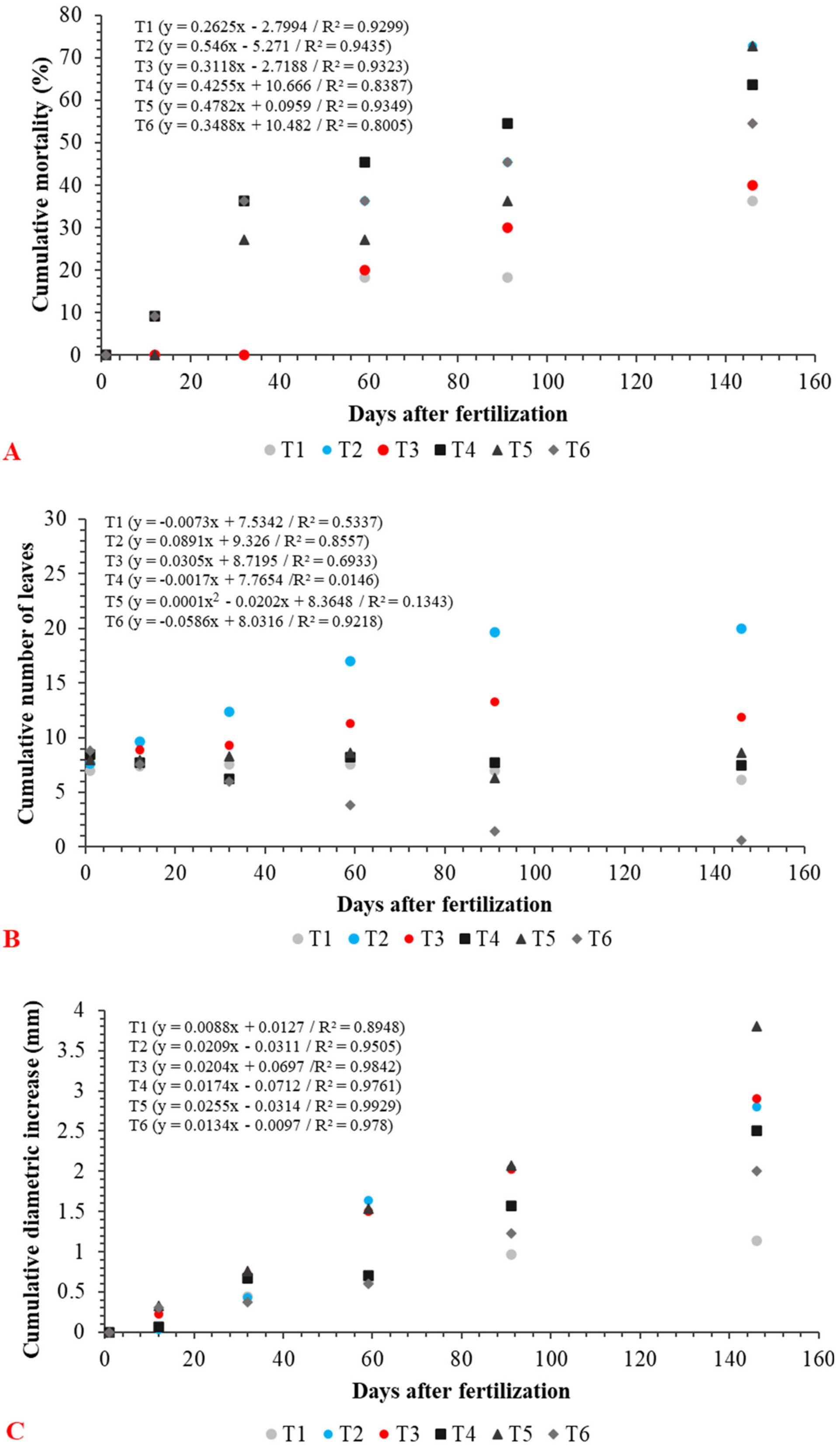

Figure 1. Growth parameters evaluated. Cumulative percent dieback (A), cumulative number of leaves (B), cumulative diameter increment (C) and cumulative height increment (D).

increase is observed until the end of the trial, with treatments T2, T3 and T4, an increase in the number of leaves after fertilization of *C. officinalis* plants until day 60, thereafter there was a decrease in the number of leaves; T1 shows a slight tendency to decrease the number of leaves. In general, T5 reached 17% more leaves with respect to the number of leaves recorded at the beginning of the trial, followed by T2 with 4%, and in treatments T1, T3, T4 and T6 a decrease of -14, -21, -3 and -10%, respectively, is reported (Figure 1B).

For the parameters of diameter increment (Figure 1C) and height increment (Figure 1D), similar

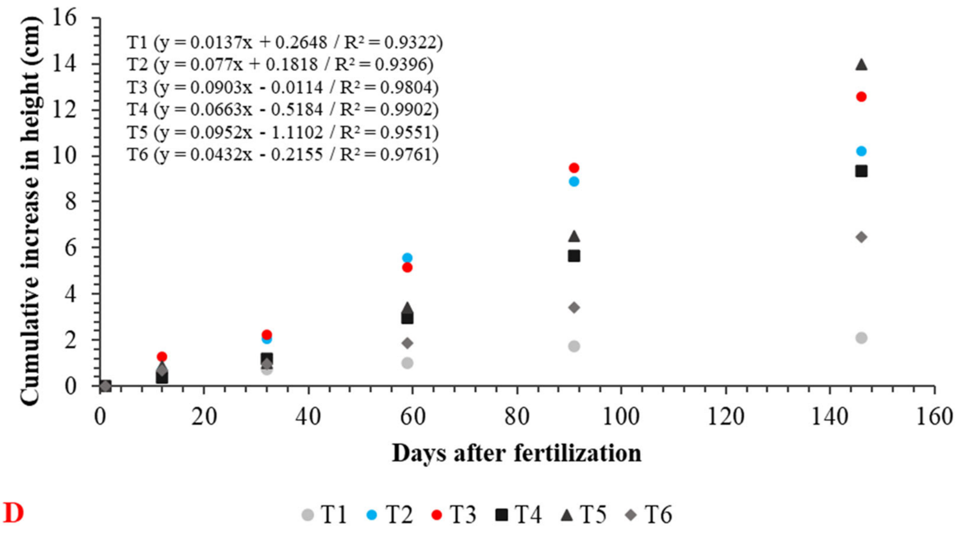

Figure 1. Continued.

Table 2. Multifactorial ANOVA with growth parameters as dependent variables.

Source	Sum of squares	Degrees of freedom	Mean square	F	P
Diameter					
Dose	15.15	5	3.03	4.59	**0.0007**
Time	103.57	5	20.71	31.35	**0.0000**
Dose x Time	12.82	25	0.51	0.79	0.7650
Residues	83.2498	126	0.66		
Total (corrected)	222.88	161			
Height					
Dose	432.36	5	86.47	16.22	**0.0000**
Time	1588.71	5	317.74	59.6	**0.0000**
Dose x Time	457.72	25	18.31	3.43	**0.0000**
Residues	735.69	138	5.33		
Total (corrected)	3165.35	173			
Mortality					
Dose	21.08	5	4.22	0.64	0.6699
Time	133.78	5	26.75	4.06	**0.0014**
Dose x Time	243.13	25	9.73	1.47	0.0686
Residues	2374.15	360	6.59		
Total (corrected)	2772.14				
Number of leaves					
Dose	153.58	5	30.72	8.45	**0.0000**
Time	25.49	5	5.09	1.4	0.2271
Dose x Time	136.63	25	5.46	1.5	0.0731
Residues	494.25	136	3.63		
Total (corrected)	811.16	171			

P-values in bold indicate significant differences between the means of each treatment.

Table 3. Differences between the means of the 6 treatments.

Treatment	Mortality (%)	Height increase (cm)	Diameter increase (mm)	Number of leaves
T1	36.36 ± 0.94e	2.11 ± 0.94c	1.14 ± 0.58b	6.14 ± 2.12b
T2	72.72 ± 6.30a	10.2 ± 6.30ab	3.8 ± 0.95a	10.33 ± 2.08a
T3	40.00 ± 4.25d	12.57 ± 4.25a	2.28 ± 1.03a	6.7 ± 2.51b
T4	63.63 ± 5.63b	9.32 ± 5.63ab	2.8 ± 1.39a	8 ± 3.79ab
T5	72.72 ± 4.75a	14.00 ± 4.75a	2.9 ± 1.24a	8.25 ± 2.22ab
T6	54.54 ± 4.99c	6.48 ± 4.99bc	2.775 ± 0.95a	8 ± 3.9ab

Lowercase letters, indicating significant differences (α = 0.05).

behaviors were observed, the highest values were observed in treatment T5, followed by T3 and T2. In both parameters, the lowest increases were recorded in treatment T1. When comparing the results of T5 with those of T1, it was observed that the values obtained in treatment T5 for diameter and height were 70 and 85% higher than the results obtained in treatment T1. These results show the effect of fertilization on the growth of *C. officinalis* plants at nursery level.

Table 2 shows the results of the multifactorial ANOVA obtained by considering the growth parameters (mortality, height, diameter and number of leaves) as dependent variables and fertilization dose and sampling time as factors. With a significance level of 0.05, the dose and sampling time have significant effects on the diameter and height increment. Of the four parameters analyzed, only for height increment the interaction between dose and time had significant effects.

Table 3 shows the statistical differences among the six treatments evaluated. It is evident that there are no differences between T2 and T5 for the mortality parameter, but there are statistical differences between these two treatments and the other four treatments. Regarding the increase in height, T5 is the one that has registered the highest value, despite this, there are no differences with treatments T4, T3 and T2; however, there are differences between T5 (higher increase in height) and T1 (lower increase in height). Regarding the increase in diameter, T5 is the treatment that obtained the best results, with significant differences between T5 and T1; similar behavior is observed in the increase in the number of leaves.

Table 4 shows the results of the chemical properties analyzed for the substrate at the end of the test. These results were correlated with the growth parameters of C. officinalis, and were obtained for the mortality variable, it appeared that there was a negative correlation with N, Mg, Ca, pH, OM, CEC and a positive correlation with P, for the height increment variable, negative correlations were shown with Mg, Ca, Ph, CEC and positive correlation with P and EC, while for the diameter increment variable, negative correlations were seen with Mg, Ca, pH and CEC, and positive correlations with P and EC (Figure 2).

Table 4. Chemical properties of the substrate used as transplanting medium of *C. officinalis* in each treatment.

Treatment	N (%)	P (ppm)	K (ppm)	Mg (meq/100g)	Ca (meq/100g)	pH	OM (%)	CEC (meq/100g)	EC (dS/m)
T1	0.35	15.50	409.50	1.77	10.52	5.82	6.97	15.20	0.15
T2	0.34	25.83	119.94	0.44	1.67	3.85	6.90	9.60	0.76
T3	0.38	22.41	189.90	0.42	1.57	3.77	7.60	9.60	2.07
T4	0.35	26.45	216.44	0.52	2.03	3.74	7.04	9.60	2.11
T5	0.35	24.89	322.01	0.58	2.52	3.83	6.97	10.40	2.84
T6	0.36	27.59	208.08	1.00	5.19	4.73	7.25	12.00	0.83

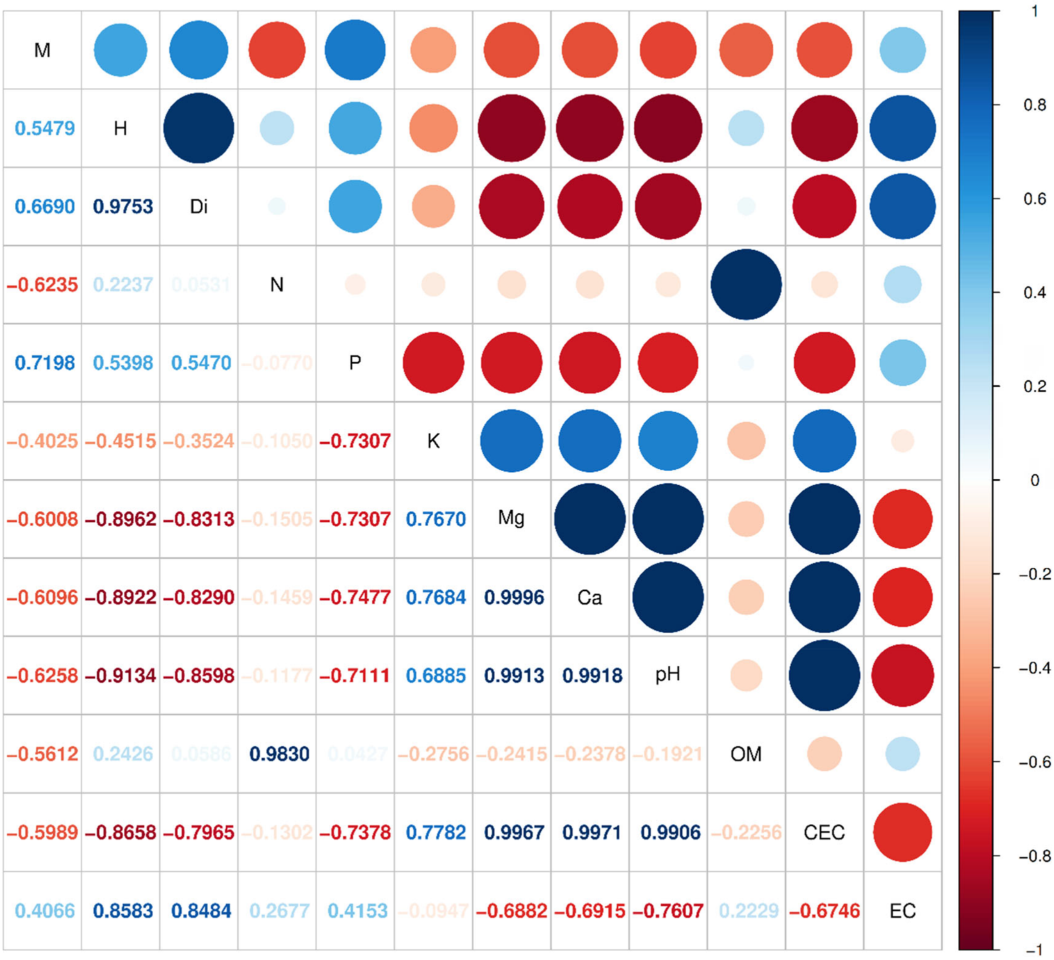

Figure 2. Correlation matrix between growth variables (M = mortality, H = height, Di = diameter) of *C. officinalis* plants obtained at the end of the trial versus the chemical properties of the substrate (N = nitrogen, P = phosphorus, K = potassium, Mg = magnesium, Ca = calcium, OM = organic matter, CEC = cation exchange capacity, EC = electrical conductivity) analyzed at the end of the trial.

Discussion

Several investigations were developed to evaluate the effect of fertilization on the growth of forest species, however, most of these investigations developed in tropical areas focused on introduced species of commercial interest (Alvarado and Raigosa 2012). This research, to the best of our knowledge, is pioneering in analyzing the response to chemical fertilization in *C. officinalis*, a species of medicinal, civic and highly threatened importance (Huamán et al. 2019; Fernandez et al. 2022), with potential commercial interest and for ecological recovery programs.

The increase in the growth of *C. officinalis* plants is relatively slow, as reported by (Fernandez-Zarate et al. 2022). However, the results obtained in this research show a significantly higher growth in the fertilized treatments with respect to the treatment that was not fertilized and regardless of the fertilization dose, diameter and height have shown linear increases until the end of the trial (six months after fertilization). In general, fertilization of *C. officinalis* seedlings at nursery level showed positive results, contributing to the increase in diameter, height and number of leaves. These positive effects could contribute to revegetation in soils with competing vegetation, as has been reported for other species and site conditions (Oliet et al. 2005; Salifu et al. 2009). The significant increase in the growth parameters evaluated could be attributed, although it was not measured in this study, to the reserve of nutrients that usually accumulate in the stems and roots of fertilized plants (Hu et al. 2015; Pokharel and Chang 2016).

The mortality rate of *C. officinalis* plants varied with the treatments; in general, fertilization contributed to an increase in seedling mortality, reaching 73% of dead plants compared to 36% mortality when no fertilizer was applied; this is the average mortality rate at the nursery level for this species (Fernandez et al. 2021). This high percentage of mortality would be associated with the mineral composition of the product used in the study, since an imbalance in nutrients usually has a negative effect on plant survival rate when there is an excess of any of them (Grzyb et al. 2013; Christophe et al. 2019).

If the nutritional distribution of the fertilizer used in this trial is observed, it is evident that it has a high nitrogen content (19%), which although it can have positive effects on the number of leaves on the plant (Onyango 2002), the excess of this nutrient could lead to an increase in the mortality rate since it tends to generate yellowing of the leaves and trigger their dehiscence and subsequent death of the plants (Westerveld et al. 2003; Perner et al. 2007).

Regarding potassium, it is associated with photosynthesis (Sardans and Peñuelas 2015), enzymatic activity and protein synthesis (Armengaud et al. 2009). It is also known that, if adequate potassium fertilization is performed, it generates protection to plants from biotic and abiotic stresses (Wang et al. 2013; Sardans and Peñuelas 2015) and with respect to magnesium, if the dose is small, it could trigger growth inhibition and tissue aging (Verbruggen and Hermans 2013), since there are stomatal limitations and low CO_2 assimilation and, with respect to phosphorus, its excess could have a defoliating effect leading to plant death (Novoa et al. 2018), this can be observed in Figure 2, since there is a high positive correlation between mortality and phosphorus content in the soil.

Conclusion

The application of inorganic fertilizer to *C. officinalis* plants was beneficial for the parameters of diameter increase, height and number of leaves; however, the same results were not obtained with the mortality rate, which was higher in those treatments in which fertilizers were applied. Since this is the first research that reports on the behavior of *C. officinalis* in response to fertilization, it is suggested to follow this line of research and analyze the response of *C. officinalis* to each nutrient separately, which could probably better explain the behavior observed in the trial. In spite of this, the results show promise in the application of fertilizers in nurseries to produce *C. officinalis* plants in order to help the recovery programs of the species.

Authors' contributions

All authors contributed substantially to the conception and design, data acquisition, or analysis and interpretation of the data; participated in the drafting of the article or in its critical revision for its important intellectual content of the version to be published; and agreed to be responsible for all aspects of the work.

Disclosure statement

The authors declare that they have no conflicts of interest.

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