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Proceeding Paper

Fertilization with ZnO and $ZnSO_4$: Mineral Analyses in *Vitis vinifera* Grapes cv. Fernão Pires †

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Abstract: Nutrition of the world population has become a concern, making research for strategies to enhance crop production necessary. Thus, the study of nutrients and the interactions between them is highly necessary since they are important for plant physiology and influence the growth of crops. Zinc is an essential micronutrient required for the normal function of plants. Its deficiency is associated with losses in yield and nutritional quality. Vine, being a crop susceptible to Zn deficits, is among the most cultivated fruit plants in the world. In this study, the reactions of the variety Vitis Vinifera Fernão Pires, located in a field in Palmela, Portugal (N 38°35′41.467″ W 8°50′44.535″), to three foliar sprays of ZnO and $ZnSO_4$ with concentrations of 150 g ha^{-1} and 450 g ha^{-1} were studied. Using an X-ray fluorescence analyzer (XRF), the mineral content of the grapes and leaves was determined, which showed increases in the content of Zn. It was found that the highest concentration (450 g ha^{-1}) of $ZnSO_4$ and ZnO, led to increases of 1.3 and 1.9-fold, respectively, compared to the control (untreated plants). Importantly, XRF analysis confirmed that the K and P contents of ZnO and $ZnSO_4$-treated plants are similar to controls, indicating that there are no significant antagonistic and/or synergistic effects. Furthermore, to study the conditions of nutrient availability in the soil, parameters such as pH, organic matter and humidity were evaluated. This work showed that fertilization with $ZnSO_4$ and ZnO was effective in increasing the concentration of Zn, without negatively affecting the contents of the crucial nutrients K and P, which is important to improve crop quality.

Keywords: nutrient's interactions; *Vitis vinifera*; Zn deficits

1. Introduction

Agricultural production is expected to increase with population growth, requiring the use of fertilizers to be sufficient in quantity and quality [1].

Fertilization is considered the most efficient method to increase crop yield and quality, particularly in fruit trees [2]. The use of fertilizers has already demonstrated results, with an increase of 50% being found in crop yields during the 20th century [3]. Soil composition must be considered for proper crop nutrition, as nutrient deficiencies occur in soils around the world [4]. These deficiencies negatively affect metabolic processes, leading to adverse changes in crop growth and development [5]. In fact, it is worth highlighting the importance of some nutrients, such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Sulfur (S), and Magnesium (Mg), Iron (Fe), Zinc (Zn), Copper (Cu), Boron (B), Manganese (Mn), Molybdenum (Mo) and Chloride (Cl), that are required for the normal function of crops [5].

In this context, Zn is one of the nutrients whose deficiency in agricultural soils is common, leading to a shortage of this micronutrient in plants [6] and, consequently, reducing growth, tolerance to stress and chlorophyll synthesis [7]. This micronutrient has important functions related to gene expression, photosynthesis, the structure of enzymes, auxin metabolism, membrane permeability and protein synthesis [8].

On an economic level, the vine is a fruit species with high importance worldwide [9], being a common target of Zn deficiency [10]. Furthermore, the uptake and availability of nutrients on this fruit tree depend on soil characteristics such as the structure, type, fertility, temperature and moisture [11].

2. Materials and Methods

2.1. Experimental Field

The workflow was implemented in a *Vitis vinifera* cv. Fernão Pires, at a field located in the region of Palmela, Portugal (N 38°35′41.467″ W 8°50′44.535″). During the reproductive cycle, the grapes were subjected to three foliar applications with $ZnSO_4$ and ZnO with concentrations of 0, 150 and 450 g ha^{-1}. Harvest was performed on the 17th of September.

2.2. Organic Matter, pH and Moisture Percentage in Soils

The determination of organic matter, pH and percentage of moisture in the experimental soil was carried out in 20 samples (about 100 g were collected from the surface to a 30 cm depth). The samples were sieved (2.0 mm mesh) to remove stones, coarse materials and other debris, then dried at 105 °C for 24 h (followed by 1 h of desiccation), and then the dry mass and the moisture percentages were determined. Afterwards, the samples were heated at 550 °C for 4 h (i.e., until constant weight), then removed from the muffle at 100 °C and desiccated until room temperature (approximately 1 h), then they were weighed again and the percentage of organic matter was determined. Following [12] the pH and electrical conductivity of the soil samples were obtained, after mixing, in the proportion of 1:2.5 (g soil mL^{-1} water milli-q), for 1 h with agitation (at 25 °C for 30 min) in a thermal bath after decanting the supernatant.

2.3. Quantification of Mineral Elements in Soils, Grapes and Leaves

The mineral contents of grapes at harvest and leaves were analyzed using an XRF analyzer (model XL3t 950 He GOLDD+) under a helium atmosphere (Niton Thermal Scientific, Munich, Germany), adapted from [13]. Previously, grapes and leaves were dried at 60 °C until they were a constant weight, then they were grounded and processed into pellets.

2.4. Statistical Analysis

Data were statistically processed, applying one-way ANOVA ($p \leq 0.05$) to determined differences, and then a Tukey's test for mean comparison (95% confidence level) was performed.

3. Results

3.1. Quantification of Nutrients in Grapes and Leaves

The Zn amount in grapes and leaves increased with the concentration in both treatments ($ZnSO_4$ and ZnO), evidencing a higher value at the maximum concentrations of

ZnO in the grapes (1.94-fold increase face to control) and $ZnSO_4$ in the leaves (9.08-fold increase face to control). In control grapes and leaves, significant differences were found compared to the concentration of 450 g ha^{-1} of both treatments (Table 1).

Table 1. Average ± S.E. (n = 3) of Zn (ppm) in fruits and leaves at harvest of *Vitis vinifera* cv. Fernão Pires. Different letters (a, b, c) indicate significant differences among treatments for grapes or leaves ($p < 0.05$).

	Control (0 g ha^{-1})	**ZnO (150 g ha^{-1})**	**ZnO (450 g ha^{-1})**	**ZnSO$_4$ (150 g ha^{-1})**	**ZnSO$_4$ (450 g ha^{-1})**
Grapes	9.12 ± 0.20 ^{c}	10.78 ± 0.13 ^{bc}	17.69 ± 1.17 ^{a}	10.10 ± 0.36 ^{bc}	12.21 ± 0.49 ^{b}
Leaves	32.76 ± 4.36 ^{c}	110.30 ± 1.39 ^{bc}	176.98 ± 32.37 ^{b}	91.75 ± 13.45 ^{c}	297.47 ± 20.03 ^{a}

Regarding the grapes, Ca and S showed lower values in the ZnO 150 g ha^{-1} treatment, being significantly different compared to the control, as for the other samples, there were no significant differences (Table 2). For P nutrient, grapes subjected to treatment did not show significant differences compared to control, although the higher amount was found in the concentrations of 450 g ha^{-1} (Table 2). Values ranged from 0.20–0.38%, 0.15–0.21% and 0.13–0.18% for Ca, S and P, respectively (Table 2). Potassium in all grape samples did not show significant differences in relation to the control, though the $ZnSO_4$ 450 g ha^{-1} treatment had a higher amount (2.16%) and the control grapes had the smallest amount (1.82%) (Table 2).

Table 2. Average ± S.E. (n = 3) of the percentage (%) of Ca, K, S and P in fruits and leaves at harvest of *Vitis vinifera* cv. Fernão Pires. Different letters (a, b, c) indicate significant differences among treatments for grapes or leaves ($p < 0.05$).

			Grapes		
Treatments	**Control (0 g ha^{-1})**	**ZnO (150 g ha^{-1})**	**ZnO (450 g ha^{-1})**	**ZnSO$_4$ (150 g ha^{-1})**	**ZnSO$_4$ (450 g ha^{-1})**
			%		
Ca	0.38 ± 0.04 ^{a}	0.20 ± 0.01 ^{b}	0.34 ± 0.04 ^{a}	0.27 ± 0.02 ^{ab}	0.31 ± 0.02 ^{ab}
K	1.82 ± 0.12 ^{a}	1.93 ± 0.02 ^{a}	1.93 ± 0.05 ^{a}	2.07 ± 0.09 ^{a}	2.16 ± 0.21 ^{a}
S	0.19 ± 0.01 ^{ab}	0.15 ± 0.00 ^{c}	0.21 ± 0.00 ^{a}	0.17 ± 0.01 ^{bc}	0.19 ± 0.01 ^{ab}
P	0.16 ± 0.01 ^{ab}	0.13 ± 0.00 ^{b}	0.18 ± 0.01 ^{a}	0.15 ± 0.01 ^{ab}	0.17 ± 0.00 ^{a}
			Leaves		
			%		
Ca	3.86 ± 0.16 ^{ab}	4.40 ± 0.02 ^{a}	4.17 ± 0.15 ^{a}	2.94 ± 0.27 ^{c}	3.26 ± 0.25 ^{bc}
K	2.17 ± 0.05 ^{a}	2.20 ± 0.20 ^{a}	2.60 ± 0.23 ^{a}	2.12 ± 0.17 ^{ab}	1.37 ± 0.12 ^{b}
S	0.87 ± 0.03 ^{ab}	0.94 ± 0.11 ^{ab}	1.06 ± 0.05 ^{a}	0.93 ± 0.15 ^{ab}	0.58 ± 0.03 ^{b}
P	0.26 ± 0.00 ^{bc}	0.32 ± 0.01 ^{ab}	0.36 ± 0.03 ^{a}	0.27 ± 0.03 ^{bc}	0.18 ± 0.01 ^{c}

Relative to leaves, a different tendency is observed for Ca, with the treatment $ZnSO_4$ 150 g ha^{-1} showing significant differences compared to control (Table 2). Concerning S in the leaves, all the analyzed grapes did not show significant differences compared to control (Table 2). The macronutrient K in the treatment $ZnSO_4$ 450 g ha^{-1} demonstrated to be significantly different compared to grapes without Zn fertilization, showing a lower value (Table 2). As for P, it showed significant differences between treatment ZnO 450 g ha^{-1} and control grapes (Table 2). In the leaves, it was observed that the treatment ZnO showed higher values for all the elements analyzed with the concentration of 450 g ha^{-1} (except for Ca), although not significant (Table 2).

3.2. Soil Parameters

Soil pH demonstrated a range approximatively between 6.4 and 7, although one sample presented more alkalinity (7.6) (Figure 1).

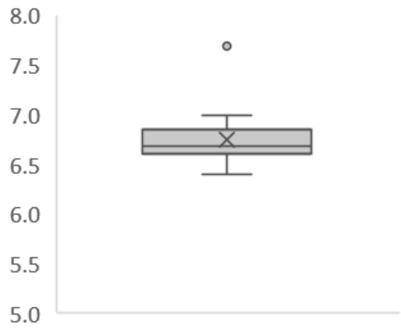

Figure 1. Boxplot of pH of soil field of *Vitis vinifera* cv. Fernão Pires (n = 20).

Regarding organic matter and moisture, all the samples analyzed did not present significant differences compared to control (Figure 2). Moisture and organic matter values varied approximately between 4–6% and 0.80–1.50%, respectively (Figure 2).

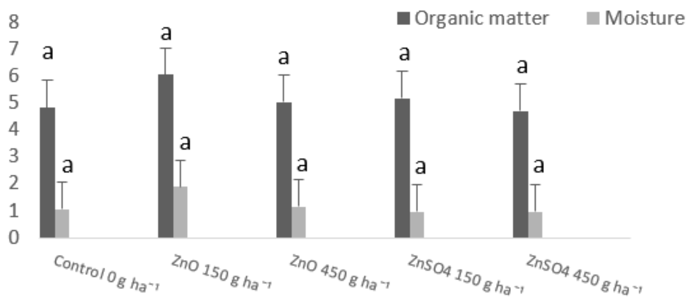

Figure 2. Average content + S.E. (n = 3) of organic matter (%) and moisture (%) from soils field of *Vitis vinifera* cv. Fernão Pires. Letter a indicate the absence of significant differences among treatments (p < 0.05).

4. Discussion

Crop deficiencies and the consequences in quality are a preoccupation nowadays, being used fertilizers, namely through foliar application once it prevents problems such as fixation and immobilization of the nutrients in soils [14,15]. Moreover, the use of foliar fertilization allows efficient absorption and translocation [15] in different phases of development and according to the needs of plants [14].

The mineral analysis of this study demonstrated a positive response, increasing the Zn amount (Table 1), through foliar fertilization with $ZnSO_4$ and ZnO, being more pronounced in the higher concentration of both treatments in leaves and grapes. However, the Zn inorganic source more often used is $ZnSO_4$ because it is more soluble in water and cheaper [16]. In this study, the treatment ZnO revealed the highest increase in Zn in Fernão Pires grapes (Table 1). Zn foliar fertilization, as observed in other studies, has benefits in the growth and development of fruit trees (i.e., mandarin, orange and grapefruit) [16], additionally reducing Zn deficiency in crops and enhancing the uptake of other nutrients, as reported in [17].

In this context, the response to treatments in the leaves was better with ZnO as it had a positive trend (except for Ca) and an inhibitory response with $ZnSO_4$ 150 and 450 g ha^{-1} was observed for Ca and K, respectively (although no significant differences were observed). As for grapes, a negative response was observed in our data for Ca and S, diminishing the concentration with the application of the lowest concentration of treatment ZnO (150 g ha^{-1}). Contrarily, a positive tendency was observed in K, S and P in grapes subjected to $ZnSO_4$ and/or ZnO fertilizers in the higher concentration (450 g ha^{-1}) (Table 2), but no significant antagonistic and/or synergistic relationships were observed. Although, in this concentration, it did not interfere significantly with these nutrients, Zn fertilization is a strategy to enhance the yields of crops and avoid the need to use more fertilizers, consequently being more sustainable for the environment [18].

On the other hand, nutrient uptake in plants is dependent on soil pH, water content and organic matter [17,19]. Yet, there are other factors in soils that interfere with the bioavailability of nutrients, those relations being a result of the combined properties of soils [20]. In this context, the organic matter and moisture data of this study showed no significant differences in the soil samples (Figure 2). Thus, these two soil parameters did not influence the differences observed with Zn fertilization in this experimental study. For the production of vines, a suitable soil pH is in the range between 5.5–8 (i.e., slightly acid and neutral), providing better growing conditions as it leads to an adequate amount of essential nutrients [21]. In the Fernão Pires field, pH presented values ranging from 6.4 to 7 (except for one sampling site with 7.6) (Figure 1). These conditions were suitable for grape production, therefore, also for the performance of this study.

5. Conclusions

The application of Zn fertilizers such as $ZnSO_4$ and ZnO at concentrations of 150 and 450 g ha^{-1} was efficient in increasing the Zn amount in Fernão Pires grapes. With ZnO fertilizer showing a greater ability to increase the Zn amount, although it is less soluble than $ZnSO_4$. Additionally, demonstrating that the highest concentration in grapes does not interfere negatively with other essential nutrients such as K, P and S, since no antagonistic or synergistic relationships were observed. Since fertilization with Zn is related to benefits in the growth and development of fruit trees, the results of this study show potential benefits in crop productivity.

Supplementary Materials: The presentation material can be downloaded at: https://www.mdpi.com/article/10.3390/IECHo2022-12512/s1.

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