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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 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 a X-ray fluorescence analyzer (XRF), the mineral content of the grapes and leaves was determined, which showed increases in the contents 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 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, have already demonstrated results, being found an increase of 50 % on 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 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 with gene expression, photosynthesis, structure of enzymes, auxin metabolism, membrane permeability and protein synthesis [8].

On an economic level, the vine is a fruit species with a high importance worldwide [9], being a common target of Zn deficiency [10]. Furthermore, the uptake and availability of nutrients on this fruit tree, depends on soil characteristics 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″ O 8°50′44.535″ W). Along 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 at 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, 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), being weighed again and determined the percentage of organic matter. With the aid of a potentiometer, 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 [12].

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

Mineral content of grapes at harvest and leaves were analyzed using an XRF analyzer (model XL3t 950 He GOLDD +) under helium atmosphere, adapted from [13]. Previously, grapes and leaves were dried at 60 °C until constant weight, 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

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 ± 0.20c	10.78 ± 0.13bc	17.69 ± 1.17a	10.10 ± 0.36bc	12.21 ± 0.49b
Leaves	32.76 ± 4.36c	110.30 ± 1.39bc	176.98 ± 32.37b	91.75 ± 13.45c	297.47 ± 20.03a

Regarding the grapes, Ca and S showed lower values in 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 didn't showed significative 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 the smallest amount (1.82 %) (Table 2).

Relatively to leaves a different tendency is observed for Ca, with the treatment $ZnSO_4$ 150 g ha^{-1} showing significative differences face to control (Table 2). Concerning to S in the leaves, all the analyzed grapes didn't show significative differences compared to control (Table 2). The macronutrient K in the treatment $ZnSO_4$ 450 g ha^{-1} demonstrated to be significatively different compared to grapes without Zn fertilization, showing a lower value (Table 2). As for P showed significative 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 450 g ha^{-1} (except for Ca), although not significative (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 ± 0.04a	0.20 ± 0.01b	0.34 ± 0.04a	0.27 ± 0.02ab	0.31 ± 0.02ab
K	1.82 ± 0.12a	1.93 ± 0.02a	1.93 ± 0.05a	2.07 ± 0.09a	2.16 ± 0.21a
S	0.19 ± 0.01ab	0.15 ± 0.00c	0.21 ± 0.00a	0.17 ± 0.01bc	0.19 ± 0.01ab
P	0.16 ± 0.01ab	0.13 ± 0.00b	0.18 ± 0.01a	0.15 ± 0.01ab	0.17 ± 0.00a
			Leaves		
			%		
Ca	3.86 ± 0.16ab	4.40 ± 0.02a	4.17 ± 0.15a	2.94 ± 0.27c	3.26 ± 0.25bc
K	2.17 ± 0.05a	2.20 ± 0.20a	2.60 ± 0.23a	2.12 ± 0.17ab	1.37 ± 0.12b
S	0.87 ± 0.03ab	0.94 ± 0.11ab	1.06 ± 0.05a	0.93 ± 0.15ab	0.58 ± 0.03b
P	0.26 ± 0.00bc	0.32 ± 0.01ab	0.36 ± 0.03a	0.27 ± 0.03bc	0.18 ± 0.01c

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).

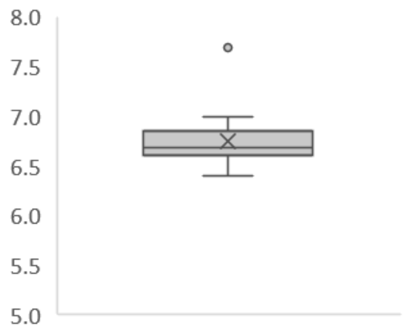

Figure 1. Boxplot of pH of soil field of *Vitis vinifera* cv. Fernão Pires (n = 20).

Regarding organic matter and moisture, all the samples analyzed, didn't presented significative differences face to control (Figure 2). Moisture and organic matter values varied approximately between 4–6 % and 0.80–1.50 % respectively (Figure 2).

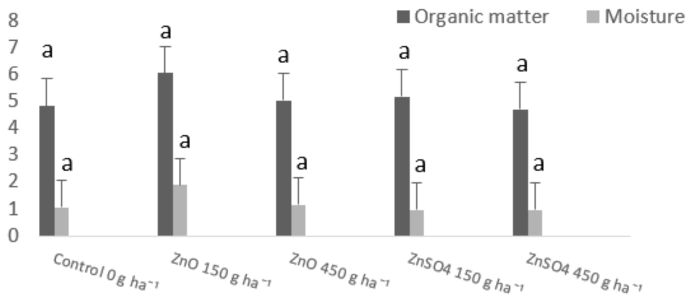

Figure 2. Average content + S.E. (n = 3) of organic matter (%) and moisture (%) from soils field of *Vitis vinifera* cv. Fernão Pires. Letter a indicate the absence of significant differences among treatments ($p < 0.05$).

4. Discussion

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

The mineral analysis of this study demonstrated a positive response increasing 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. Although, Zn inorganic source more used is $ZnSO_4$, because 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 have benefits in 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 with $ZnSO_4$ 150 and 450 g ha^{-1} an inhibitory response was observed for Ca and K, respectively (although no significant differences were observed). As for grapes, it was observed in our data for Ca and S, a negative response, diminishing the concentration with the application of the lowest concentration of treatment ZnO (150 g ha^{-1}). Contrarily, it was observed a positive tendency 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 significative antagonistic and/or synergistic relationships were observed.

Although in this concentration didn't interfere significatively 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 to 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 bioavailability of nutrients, being those relations, a result of a 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. Regarding the pH, for the production of vines a suitable soil has a 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]. Which in Fernão Pires field, pH presented values ranging from 6.4 to 7 (except for one sampling site, with 7.6) (Figure 1), being these conditions suitable for the grapes production, therefore also for the performance of this study.

5. Conclusions

Application of Zn fertilizers such as $ZnSO_4$ and ZnO at concentration of 150 and 450 g ha^{-1} was efficient increasing the Zn amount in Fernão Pires grapes. With ZnO fertilizer showing 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.

6. Patents

Author Contributions: Conceptualization, P.L., and F.C.L.; methodology, P.L, and F.C.L.; formal analysis, D.D., A.R.F.C., C.C.P., I.C.L., and A.C.M.; resources, J.C.R., P.S.C., I.P.P., J.N.S., M.M.S., P.L., M.S., F.H.R., M.F.P. and F.C.L.; writing—original draft preparation, D.D.; writing—review and editing, D.D. and F.C.L.; supervision, F.C.L.; project administration, F.C.L.; funding acquisition, F.C.L. All authors have read and agreed to the published version of the manuscript.

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