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Can Precision Agriculture Be Used in the Management of a Fe and Zn Biofortification Workflow in Organic Tomatoes (*Lycopersicum esculentum* L.)? †

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Abstract: It is expected that the population worldwide might exceed 9 billion by 2050, therefore it being imperative to increase food production. As such, the development of smart farming technology is an important key food production issue. In fact, through the use of UAVs (Unmanned Aerial Vehicles), it is possible to create normalized difference vegetation index (NDVI) maps, that can indicate factors, such as health and vegetation vigor. In this context, this study aimed to assess the state of three tomato varieties (beef heart, "chucha", and apple) in the framework of a biofortification workflow with Fe and Zn, following an organic production mode. In a tomato experimental production field (GPS coordinates—39°41′48.517″ N; 8°35′45.524″ W), six foliar sprayings were carried out during the production cycle, with a mix of Zitrilon (15%) (0.40 and 1.20 kg·ha^{-1}) and Maxiblend (1 and 4 kg·ha^{-1}). NDVI was determined 7 days before the first foliar spraying and showed a maximum of 0.86 (on a scale from −1 to 1). After the 3rd foliar spraying, no changes were detected in the color of freshly harvest tomatoes (assessed through spectrophotometric colorimeter), but an increase of Fe and Zn content was found in the leaves, and of Zn in tomatoes themselves (except in "chucha" variety). The use of precision agriculture techniques in correlation with the other analyses is discussed.

Keywords: biofortification; Iron; *Lycopersicum esculentum* L.; NVDI; organic tomato production; Zinc

1. Introduction

The worldwide population is expected to exceed the 9 billion by 2050 [1], and, as such, food productions must increase by 25–70% to be able to feed the future world population [2]. Agriculture has changed over the years, and the digital era today is considered the future of this sector. In fact, the development of smart farming technology has afforded the ability to continuously monitor the states of plants, soils, and the needs for productions inputs (such as water) [3], thus being an important tool for food production. UAVs (Unmanned Aerial Vehicles) are being used in monitoring and measuring bio-physical parameters [4]. Nevertheless, through the data obtained by UAVs, it is possible to create NDVI (Normalized Difference Vegetation Index) maps (ranging from -1 to 1) [5], thus being one of the most used and implemented calculated indices. This type of index can characterize health and vegetation vigor [6].

The lack of essential nutrients, such as Fe and Zn, in human diets is a current global problem [7], which can lead to the development of several pathologies, namely anemia (for Fe deficiency) [8] or problems related to the immune system, gastrointestinal, central nervous, and reproductive system (for Zn deficiency) [9].

Zinc supports normal growth and development during different stages of life (mainly during pregnancy, childhood, and adolescence). In fact, the daily adequate intake for pregnant and lactating women ranges between 11–13 mg; from birth to 8 years, it varies between 2–5 mg; and, for individuals from 9 years of age or older, the daily dose varies between 8–11 mg (dependent upon gender) [10]. On the other hand, Fe is an essential component of hemoglobin, supports muscle metabolism, and plays an essential role in physical growth and cellular functioning. Iron daily adequate intake varies between males, females, those who are pregnant, and those who are lactating, thus large quantities being needed during pregnancy (27 mg) [11]. As such, since edible agriculture products are the main source of minerals [12], biofortification has been carried out over the years, aiming to attain biofortified food crops [13], where contents of target minerals increase in the edible part of plants.

The tomato is considered one of the most popular and widely consumed vegetables worldwide [14]; thus, organic food consumption worldwide continues to grow, and organic produce counts on a growing market [15]. Furthermore, the restriction of applied products for pest and disease control [16] implies a more accurate monitorization of crops to avoid total losses. In this context, this study aimed to assess, through precision agriculture, the state of three tomato varieties (beef heart, "chucha", and apple) biofortified with Fe and Zn, following an organic production mode.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental tomato-growing field, located in Western Portugal (39°41′48.517″ N; 8°35′45.524″ W), was used to growth three tomato varieties (beef heart, "chucha", and apple) (*Lycopersicum esculentum* L.), following an organic production mode. During the agricultural period, from 22 May (planting date) to 3 September of 2020 (harvest date), air temperatures reached an average daily of 30.6 and 13.3 °C (with minimum and maximum values varying between 5.3 and 40.6 °C, respectively). Foliar spraying with Fe and Zn was carried out with two treatments during the production cycle, with six foliar sprays (with 10–11 days interval). Treatments were carried out with a mix of two products (Zitrilon, 15%, and Maxiblend), in which treatment 1 (T1) corresponds to a mix of 0.40 $kg \cdot ha^{-1}$ Zitrilon (15%) and 1 $kg \cdot ha^{-1}$ Maxiblend and treatment 2 (T2) corresponds to a mix of 1.20 $kg \cdot ha^{-1}$ Zitrilon (15%) and 4 $kg \cdot ha^{-1}$ Maxiblend. Control plants were not sprayed at any time with Fe and Zn. Each treatment was performed in quadruplicate.

2.2. NDVI (Normalized Difference Vegetation Index) in the Experimental Field

The experimental field was flown over once with a UAV (Unmanned Aerial Vehicle), equipped with altimetric measurement sensors and synchronized by GPS. The flight was performed on 26 June (seven days before the 1st foliar spraying) to characterize vegetation indexes and to monitor differences in vigor between control and sprayed plants. The images were processed in ArcGIS Pro, and the NDVI maps were obtained.

2.3. Iron and Zinc Contents in Leaves and Zinc Content in Tomatoes

Iron and Zinc contents in leaves and Zn content in tomatoes were determined after the 3rd foliar spraying (23 July), after being cut, dried (at 60 °C, until constant weight), and grounded, using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, according to Reference [17].

2.4. Colorimetric Parameters

Colorimetric parameters were determined in fresh tomatoes per treatment with a scanning spectrophotometric colorimeter, according to Reference [18]. Measurements were carried out in quadruplicate.

2.5. Statistical Analysis

Statistical analysis was carried out using a One-Way ANOVA to assess differences among treatments in cv. Picasso, followed by a Tukey's for mean comparison. A 95% confidence level was adopted for all tests.

3. Results

Concerning the management of Fe and Zn biofortification workflow in organic tomatoes, the NDVI map was obtained 7 days after the 1st foliar spraying (Figure 1), with the aim of verifying if the culture was in good health conditions to be biofortified. In fact, the NDVI map ranged between 0.19 and 0.86, corresponding with the lower NDVI in the soil, since the tomatoes plants were in an early vegetative state.

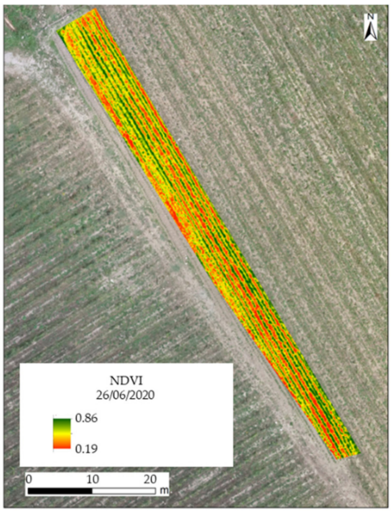

Figure 1. NDVI (Normalized Difference Vegetation Index) map in plants of *Lycopersicum esculentum* L. (considering the three varieties), 7 days before the 1st foliar spraying with Fe and Zn.

Nevertheless, from the NDVI map, the minimum, maximum, and average of NDVI was calculated (Table 1). As such, the average of NDVI was 0.44 at 7 days before the 1st foliar spraying with Fe and Zn.

Table 1. NDVI ((Normalized Difference Vegetation Index) of the three varieties of *Lycopersicum esculentum* L. (obtained on 26 June 2020), at 7 days before the 1st foliar spraying with Fe and Zn.

Minimum NDVI	Maximum NDVI	Average NDVI	SD
0.19	0.86	0.44	0.15

The colorimetric analysis of tomatoes after the 3rd foliar spraying with Fe and Zn showed the highest value at 650 nm, which corresponds to the red color (Figure 2). Only

T2 treatment of the apple variety showed higher transmittance compared to the control and T1 treatment.

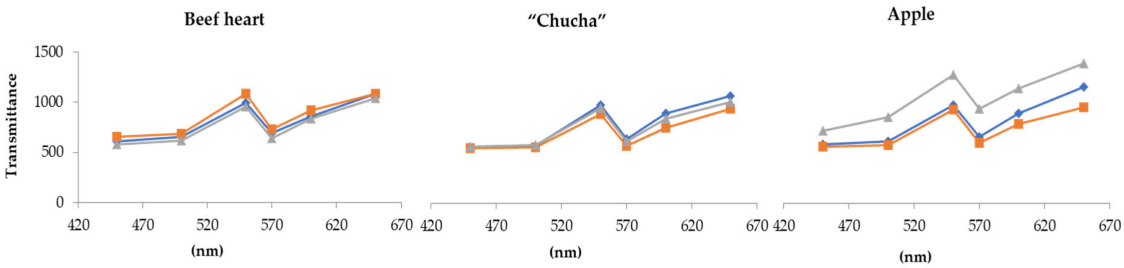

Figure 2. Visible spectra showing the average of transmittance (n = 4) in tomatoes of *Lycopersicum esculentum* L. of the three varieties, after the 3rd foliar spraying (● Control, ■ T1, and ▲ T2).

Mineral content of leaves and tomatoes was assessed after the 3rd foliar spraying (Table 2). Relative to the control leaves, T2 showed the highest content of Fe and Zn, followed by T1 in "chucha" and apple varieties. However, in the beef heart variety, the Zn content in the leaves only showed a higher content than the control in T2 treatment. In the apple variety, the Zn content in the tomatoes was significantly higher in T2 treatment. Yet, in the beef heart variety, all the treatments with Fe and Zn showed higher content than the control, although T1 showed a significantly higher content of Zn compared to T2. At this stage of the biofortification process, the "chucha" variety did not show a higher content of Zn compared to the control tomatoes.

Table 2. Mean values ± S.E. (n = 4) of Fe and Zn in leaves and Zn in tomatoes of *Lycopersicum esculentum* L. (beef heart, "chucha", and apple), after the 3rd foliar spraying.

		Leaves		Fruits
Variety	**Treatments**	**Fe (ppm)**	**Zn (ppm)**	**Zn (ppm)**
Beef heart	Control	<50	74.92b ± 1.79	38.59c ± 0.43
	T1	<50	62.29c ± 3.97	77.16a ± 0.18
	T2	<50	200.8a ± 1.96	63.12b ± 1.57
"Chucha"	Control	<50	61.67c ± 0.99	30.30a ± 1.98
	T1	140.2b ± 7.94	140.0b ± 3.14	28.76a ± 1.32
	T2	255.0a ± 10.5	167.0a± 2.26	13.71b ± 0.86
Apple	Control	73.90c ± 10.4	54.23c ± 0.70	30.35b ± 2.71
	T1	113.7b ± 3.84	121.7b ± 1.64	33.27b ± 1.43
	T2	273.9a ± 8.55	285.3a ± 2.57	64.32a ± 1.64

Different letters indicate significant differences, of each variety, between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray was carried out with two concentrations (T1 and T2). Control was not sprayed.

4. Discussion

Organic production uses lower levels of pesticides [16], thus it being important to monitor the culture. As such, before implementation of the biofortification workflow, it was necessary to ensure the best conditions of the culture. The average of NDVI was 0.44, and the maximum was 0.86 (Figure 1; Table 1). Regarding the average of NDVI, moderate values (between 0.2 to 0.5) were found [5], corresponding to the early vegetative state of tomato plants and the spacing between plants. Additionally, the maximum NDVI of the culture was 0.86, corresponding to high values (i.e., varying between 0.6 to 0.9) [5]. As such, the use of this parameter in the culture revealed that the culture had a healthy phenological development, allowing the implementation of the biofortification workflow.

In the middle of the biofortification workflow (after the 3rd foliar spraying with Fe and Zn), the color of the tomatoes (control and sprayed with Fe and Zn—T1 and T2) was assessed (Figure 2). All the treatments of the three varieties showed a highest value at

650 nm, corresponding to the red color [18] and, thus, being an indicator of high lycopene content [19]. Therefore, the use of this technology allowed an objective definition of the maturation state of the fruits, since red is an indication of 10 times more lycopene content than yellow [20].

Nevertheless, the mineral content of the leaves, after the 3rd foliar spraying (Table 2), pointed to the fact that, after foliar spraying, the "chucha" and apple varieties became biofortified with Fe and Zn (relative to the control, revealing a higher content in T2, followed by T1). However, in the beef heart variety, Zn content only revealed higher content than the control T2, which may be due to the heterogeneity during foliar spraying or because the accumulation of minerals varies depending on the genotype [20].

Despite the heterogeneity of tomato genotypes, it is crucial to increase the contents of healthy compounds in the fruits [20]. In this context, it was found that, in the middle of the implementation of the biofortification workflow, Fe content was under the limits of detection, whereas Zn content revealed different accumulation patterns (Table 2). The apple variety, relative to the control, only showed a significantly higher content of Zn in T2, whereas beef heart revealed significantly higher contents of Zn in T1 and T2 (Table 2). Moreover, the "chucha" variety at this stage of the biofortification process did not reveal a biofortification pattern; nevertheless, it should be noted that Zn contents in tomatoes can be dependent of the maturation of the fruit and the variety [21].

5. Conclusions

Through the use of cameras, coupled to UAV, it was possible to obtain NDVI values and assess the vegetative stage of the different varieties of the tomato culture before the implementation of the biofortification workflow. In fact, the use of smart farm techniques, before biofortification, can help in the management of the culture and decision-making in real time, namely by assessing the organic culture's health before foliar spraying.

Considering this technical workflow of foliar spraying with Fe and Zn in an organic production mode, it was observed that the content of these minerals in leaves and fruits varied among tomato varieties. The apple variety showed better content relative to beef heart and "chucha" varieties. Nevertheless, foliar spraying with Fe and Zn can increase these chemical elements in tomato leaves and fruits (without major changes in the color of fruits).

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECAG2021-09662/s1.

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