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Can natural Fortification Increase Fe and Zn Content in Organically Grown Tomatoes? †

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Abstract: Natural fortification can be used to increase the mineral content of the edible part of plants. In horticultural crops, foliar fertilization is used extensively, being a way to provide nutrients through leaves (a faster way compared to soil applications). Moreover, Fe and Zn are two important nutrients for plant growth and development, despite the low kinetic mobility. As such, considering the importance of Fe and Zn in plants and the fact that tomato is one of the most consumed horticultural crops worldwide, this study aimed to verify if in the middle of a biofortification process (after two foliar applications) Fe and Zn content in tomatoes of *Solanum lycopersicum* (beef heart variety-also known as Coeur de Boeuf) organically grown can improve. The experimental field was selected and followed the protocols for tomato growth in accordance with the organic production mode. Two foliar applications were carried out during the production cycle, with a mix of two products of Fe and Zn (Zitrilon–15% and Maxiblend) with two concentrations (treatment 1 and treatment 2, corresponding to a mix of Ziltrilon and Maxiblend of 0.40 and 1 kg·ha^{-1} and 1.20 and 4 kg·ha^{-1}). Through X-ray fluorescence using a XRF analyzer under He atmosphere, leaves of tomatoes submitted to the biofortification process showed an increase of 76.9% of Fe content and double Zn content, in treatment 2. However, treatment 1 only showed increases in Zn content (by 75.5% compared to control). Regarding tomatoes fruits, treatment 2 showed an increase of 7% of Zn content, relative to control content. Naturally enriched tomatoes with Fe and Zn showed minor changes in colorimetric parameters (Chroma and Hue) and no significant differences in L parameter (brightness/luminosity), regarding control. Additionally, biofortification did not affect tomatoes height and diameter at this stage of development, varying between 75.7–84.3 mm and 76.7–93.3 mm, respectively. In conclusion, two foliar sprays of Fe and Zn can improve tomatoes and leaves content under organic production practices without triggering toxicity to the plants and not affecting tomatoes height and diameter, and only presented some minor changes in color parameters (CieLab scale).

Keywords: biofortification; natural enrichment with iron and zinc; organic tomato productions; *Solanum lycopersicum*

1. Introduction

Tomato (*Lycopersicum esculentum*) is considered internationally one of the most important agricultural food crop [1] and the second most important vegetable crop [2]. For 2020, worldwide estimate production of tomato is currently 38.5 million tonnes, an increase of 3.1% compared to 2019's production [3]. Additionally, agriculture is facing huge challenges namely, related to natural resources, production methods, quality, and safety of food products [4]. In fact, modern consumers are more attentive to food nutrition and to practice healthier eating [4] and are willing to pay extra prices for organic production considering the environmental benefits and the association with sustainable foods [5]. Compared to intensive agriculture, that negatively affects the environment and human health, organic production is considered a more sustainable farming method [4]. Yet, the feasibility of organic production varies across the countries, mainly because of the weather, pest, and economic challenges [5]. In 2017, organic farming in Portugal was carried out on 253,786 hectares of agricultural land (more 26.4% than in 2012) [4], yet despite the fast evolution, the market is still a niche [6]. Regarding the mineral content, agronomic biofortification can be used to increase mineral content in the edible part of plants and is usually used in horticultural crops (as tomato) through leaves application [7]. This strategy (agronomic biofortification) can be used to suppress the lack of essential nutrients being considered a current global problem [8]. The lack of essential nutrients, as Fe and Zn, can lead to several pathologies, namely can lead to anemia [9] and problems related to immune and reproductive system, respectively [10]. Also, Fe and Zn are two important nutrients for plant growth and development, having a low kinetic mobility [11,12]. Moreover, considering the importance of Fe and Zn in humans and in plants, and the role of tomato in human diet, this study aimed to monitor and verify in a commercial variety (beef heart variety) if after two foliar applications with Fe and Zn (in the middle of a biofortification process) the content of both elements can improve following an organic production mode.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental tomato-growing field, located in the Western of Portugal (Ourém) (GPS coordinates: 39° 41′ 23 48,517″ N; 8°35′ 45,524″), was used to growth one tomato beef heart variety (*Lycopersicum esculentum* L.), following an organic production mode. Planting date was on 12 June and harvest date on 4 October 2019 (biofortification itinerary was completed after four foliar sprays that were carried out during the agricultural period with 10–11 days interval). The first foliar spray occurred on 5 September and the second 11 days after. The biofortification was performed with a mix of two products (Zitrilon (15%) and Maxiblend), in which treatment 1 (T1 or low mix) corresponds to a mix of 0.40 kg·ha^{-1} Zitrilon (15%) and 1 kg·ha^{-1} Maxiblend and treatment 2 (T2 or high mix) corresponds to a mix of 1.20 kg·ha^{-1} Ziltrilon (15%) and 4 kg·ha^{-1} Maxiblend. Both products (Ziltrilon (15%) and Maxiblend) can be used in organic farming. Zitrilon (15%) can be used as a foliar fertilizer and applied in tomato crop. This product is a concentrated Zn fertilizer with 15% in chelated form (EDTA). Maxiblend can be foliar applied to crops and is a commercial product mostly constituted by Fe (5.3%) and other micronutrients (as Mn, Zn, Cu, B, Mo, and Mg). Each treatment was performed in quadruplicate and control plants were not sprayed at any time with Fe and Zn.

2.2. Mineral Content in Leaves and Tomatoes

Mineral content was carried out by X-ray fluorescence, using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, in tomatoes and leaves after two foliar sprays, following [13,14].

2.3. Quality Parameters

Colorimetric parameters, using fixed wavelength, followed [15] were carried out in four randomized fresh tomatoes of *Solanum lycopersicum* (beef heart variety). Height and diameter were measure in four randomized tomatoes per treatment.

2.4. Statistical Analysis

Data were statistically analyzed using a One-Way ANOVA to assess differences among treatments in beef hear variety, followed by a Tukey's for mean comparison. A 95% confidence level was adopted for all tests.

3. Results

Iron and zinc content were assessed in leaves and tomatoes (*Solanum lycopersicum*) of beef heart variety after two foliar sprays with a mix of Fe and Zn (Table 1). In leaves, regarding control, treatment 2 showed an increase of 76.9% of Fe and treatment 1 showed a lower content. Regarding Zn, treatment 1 showed an increase of 75.5% and treatment 2 double the content relative to control content. In leaves, Zn content was higher in the bio-fortified treatments applied. In tomatoes, Fe content was below the device detention limit value (<35 ppm) and regarding Zn, only treatment 2 showed a higher content than control (with an increase of 7%).

Table 1. Mean values ± S.E. (n = 4) of Fe and Zn in dry leaves and in dry tomatoes of *Lycopersicum esculentum* (beef heart variety), after the 2nd foliar spraying with Fe and Zn. Different letters indicate significant differences, between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray was carried out with two concentrations (T1 or low mix and T2 or high mix). Control was not sprayed.

		Leaves		Tomatoes	
Variety	**Treatments**	**Fe (ppm)**	**Zn (ppm)**	**Fe (ppm)**	**Zn (ppm)**
Beef heart	Control	195b ± 5.9	224c ± 1.4	<35	12.9a ± 0.3
	T1 (low mix)	115c ± 3.2	393b ± 2.8	<35	10.2b ± 1.2
	T2 (high mix)	345a ± 4.0	468a ± 2.2	<35	13.8a ± 1.2

The colorimetric analysis of tomatoes after two foliar sprays with Fe and Zn showed significant differences between control and the two treatments applied (T1 (low mix) and T2 (high mix)) in Chroma, and Hue parameters (Table 2). Control showed higher values in Hue parameter and a lower in Chroma parameter. Additionally, treatment 2 presented a higher value in Chroma and a lower in Hue parameter.

Table 2. Mean values ± S.E. (n = 4) of colorimetric parameters (L, Chroma, and Hue) in fresh tomatoes of *Lycopersicum esculentum* (beef heart variety), after the 2nd foliar spraying with Fe and Zn. Different letters indicate significant differences, between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray was carried out with two concentrations (T1 or low mix and T2 or high mix). Control was not sprayed.

Variety	**Treatments**	**L**	**Chroma**	**Hue**
Beef heart	Control	43.7a ± 1.2	35.5c ± 1.9	65.5a ± 1.32
	T1 (low mix)	43.0a ± 1.1	37.8ab ± 1.3	54.6b ± 2.0
	T2 (high mix)	43.7a ± 0.8	43.8a ± 1.9	47.4c ± 0.7

Independently of higher contents of Fe and Zn, in tomatoes in the middle of a biofortification process, height and diameter did not vary significantly (Table 3). However, treatment 1 showed higher height and diameter compared to control and treatment 2.

Table 3. Mean values ± S.E. (n = 4) of height and diameter (mm) in leaves and Zn in tomatoes of *Lycopersicum esculentum* (beef heart variety), after the 2nd foliar spraying with Fe and Zn. Letter a indicate no significant differences, between treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$). Foliar spray was carried out with two concentrations (T1 or low mix and T2 or high mix). Control was not sprayed.

Variety	Treatments	Height (mm)	Diameter (mm)
Beef heart	Control	75.7a ± 0.9	83.7a ± 7.0
	T1 (low mix)	84.3a ± 8.7	93.3a ± 8.3
	T2 (high mix)	81.0a ± 3.1	76.7a ± 5.7

4. Discussion

Globally organic agriculture is growing importance, agreeing with the new need of the society in, not only in protecting the environment but also, in improving the quality of food production [4]. As such, Fe and Zn (being two essential nutrients both for humans and plants) content were assessed in leaves and tomatoes of a commercial variety (beef heart variety) after two foliar sprays with a mix of Fe and Zn in tomatoes plants organically grown (Table 1). Regarding Fe in leaves, treatment 2 (T2) or high mix was the only treatment that showed higher content compared to control. Treatment 1 showed a lower content than control and can be due to poor foliar application, external factors when applying the mix or the fact that no biofortification occurred at this stage with the applied mixture (it is not possible to confirm as the Fe content in tomatoes was lower than the detection limit of the device). In the other hand, Zn content improved in leaves with both mixtures applied, yet increased with the increasing of the applied concentration (high mix > low mix), despite the limited mobility in leaves [16]. However, in tomatoes, T1 or low mix showed a lower content of Zn than control, probably due to the low mobility within the plant [11], yet T2 or high mix showed a not significantly increase compared to control. This can be since Zn content in tomatoes can be dependent of the maturation of the fruit and variety and at this stage the tomatoes weren't mature [17]. Regarding the colorimetric parameters there were some minor changes in Chroma and Hue parameters (Table 2). In fact, color is one of the most relevant quality parameters in tomatoes, affecting consumers acceptability [18,19]. The minor changes observed in both parameters (Chroma and Hue) is due mainly to changes in a* parameter (data not shown) that showed higher representability of red color (more mature tomatoes) in T2 followed by T1 and lastly to control. As such, T2 showed more lycopene content than T1 and control at this stage of tomato development [18,19]. In addition, control tomatoes showed a higher Hue, corresponding to a higher saturation of color. Regarding the height and diameter of tomatoes, our data showed similar values to those obtained in a previous study carried out with the same variety at harvest [20].

5. Conclusions

Through foliar spraying with Fe and Zn fertilizers, at concentrations reported in this study (T1 or low mix and T2 or high mix), tomatoes and leaves content can be improved under organic production practices. Yet, better results (higher Fe and Zn content) were obtained with the higher mix applied (T2 or high mix) at the middle of the biofortification process and without triggering toxicity to the plants. Additionally, this improve of Fe and Zn content did not affect tomatoes height and diameter and only showed some minor changes regarding color (Chroma and Hue parameters).

Supplementary Materials: Not applicable.

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