biology and life sciences forum

MDPI

Proceeding Paper

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

Ana Rita F. Coelho [1,2,*], Diana Daccak [1,2], Inês Carmo Luís [1,2], Ana Coelho Marques [1,2], Cláudia Campos Pessoa [1,2], Maria Manuela Silva [2,3], Manuela Simões [1,2], Fernando H. Reboredo [1,2], Maria F. Pessoa [1,2], Paulo Legoinha [1,2], José C. Ramalho [2,4], Paula Scotti Campos [2,5], Isabel P. Pais [2,5], José N. Semedo [2,5] and Fernando C. Lidon [1,2]

1 Earth Sciences Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; d.daccak@campus.fct.unl.pt (D.D.); idc.rodrigues@campus.fct.unl.pt (I.C.L.); amc.marques@campus.fct.unl.pt (A.C.M.); c.pessoa@campus.fct.unl.pt (C.C.P.); mmsr@fct.unl.pt (M.S.); fhr@fct.unl.pt (F.H.R.); mfgp@fct.unl.pt (M.F.P.); pal@fct.unl.pt (P.L.); fjl@fct.unl.pt (F.C.L.)
2 GeoBioTec Research Center, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; abreusilva.manuela@gmail.com (M.M.S.); cochichor@mail.telepac.pt (J.C.R.); paula.scotti@iniav.pt (P.S.C.); isabel.pais@iniav.pt (I.P.P.); jose.semedo@iniav.pt (J.N.S.)
3 ESEAG-COFAC, Avenida do Campo Grande 376, 1749-024 Lisboa, Portugal
4 Plant Stress & Biodiversity Lab, Centro de Estudos Florestais (CEF), Instituto Superior Agronomia (ISA), Universidade de Lisboa (ULisboa), Quinta do Marquês, Av. República, 2784-505 Oeiras, Portugal
5 INIAV, Instituto Nacional de Investigação Agrária e Veterinária, 2784-505 Oeiras, Portugal
* Correspondence: arf.coelho@campus.fct.unl.pt; Tel.: +351-212-948-573
† Presented at the 1st International Electronic Conference on Horticulturae, 16–30 April 2022; Available online: https://sciforum.net/event/IECHo2022/.

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 whether, 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 the protocols for tomato growth were followed 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 tomato 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), relative to control. Additionally, biofortification did not affect the 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 tomato and leaf content under organic production practices without triggering toxicity to the plants or affecting tomatoes height and diameter, and only minor changes in color parameters were presented (CieLab scale).

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

check for updates

Citation: Coelho, A.R.F.; Daccak, D.; Luís, I.C.; Marques, A.C.; Pessoa, C.C.; Silva, M.M.; Simões, M.; Reboredo, F.H.; Pessoa, M.F.; Legoinha, P.; et al. Can Natural Fortification Increase Fe and Zn Content in Organically Grown Tomatoes? *Biol. Life Sci. Forum* **2022**, *16*, 26. https://doi.org/10.3390/IECHo2022-12504

Academic Editor: Yuyang Zhang

Published: 15 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Tomato (*Lycopersicum esculentum*) is considered internationally one of the most important agricultural food crops [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 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 practicing 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, which 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 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 (26.4% more 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 (such as tomato) through leaf 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, such as Fe and Zn, can lead to several pathologies, namely anemia [9] and problems related to immune and reproductive system, respectively [10]. Additionally, 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 tomatoes in human diet, this study aimed to monitor and verify in a commercial variety (beef heart variety) whether, after two foliar applications with Fe and Zn (in the middle of a biofortification process), the content of both elements could improve following an organic production mode.

2. Materials and Methods

2.1. Biofortification Itinerary

The experimental tomato-growing field, located in the west of Portugal (Ourém) (GPS coordinates: 39° 41′ 23 48,517″ N; 8°35′ 45,524″), was used to grow one tomato beef heart variety (*Lycopersicum esculentum* L.), following an organic production mode. Planting date was on 12 June and harvest date was 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 applied through foliar spraying 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, following [15], were carried out in four randomized fresh tomatoes of *Solanum lycopersicum* (beef heart variety). Height and diameter were measured 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 heart variety, followed by a Tukey's test 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 doubled the content relative to control content. In leaves, Zn content was higher in the biofortified 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	195 b ± 5.9	224 c ± 1.4	<35	12.9 a ± 0.3
	T1 (low mix)	115 c ± 3.2	393 b ± 2.8	<35	10.2 b ± 1.2
	T2 (high mix)	345 a ± 4.0	468 a ± 2.2	<35	13.8 a ± 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 the hue parameter and a lower value in the chroma parameter. Additionally, treatment 2 presented a higher value in chroma and a lower 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	43.7 a ± 1.2	35.5 c ± 1.9	65.5 a ± 1.32
	T1 (low mix)	43.0 a ± 1.1	37.8 ab ± 1.3	54.6 b ± 2.0
	T2 (high mix)	43.7 a ± 0.8	43.8 a ± 1.9	47.4 c ± 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 indicates 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	75.7 a ± 0.9	83.7 a ± 7.0
	T1 (low mix)	84.3 a ± 8.7	93.3 a ± 8.3
	T2 (high mix)	81.0 a ± 3.1	76.7 a ± 5.7

4. Discussion

Globally, organic agriculture is growing in importance, agreeing with the new need of the society to not only protect the environment but also improve the quality of food production [4]. As such, Fe and Zn (being two essential nutrients both for humans and plants) contents 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, which may 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 impossible to confirm, as the Fe content in tomatoes was lower than the detection limit of the device). On 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 significant increase compared to control. This may be since Zn content in tomatoes can be dependent of the maturation of the fruit and variety and at this stage the tomatoes were not 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 consumer acceptability [18,19]. The minor changes observed in both parameters (chroma and hue) are 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 the 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), tomato and leaf 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, without triggering toxicity to the plants. Additionally, this improvement 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: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECHo2022-12504/s1.

Author Contributions: Conceptualization, A.R.F.C. and F.C.L.; methodology, F.C.L.; software, A.R.F.C.; formal analysis, A.R.F.C., D.D., I.C.L., A.C.M. and C.C.P.; investigation, A.R.F.C., D.D., I.C.L., A.C.M. and C.C.P.; resources, M.M.S., M.S., F.H.R., M.F.P., P.L., J.C.R., P.S.C., I.P.P. and J.N.S.; writing—original draft preparation, A.R.F.C.; writing—review and editing, 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.

Funding: This work received funding from PDR2020-101-030701 and Fundação para a Ciência e Tecnologia, I.P. (FCT), Portugal, through the research units UIDP/04035/2020 (GeoBioTec) and UIDB/00239/2020 (CEF).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Ana Rita Marques from Quinta do Montalto, for technical assistance in the production field.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Ilahy, R.; Tlili, I.; Helyes, L.; Siddiqui, M.W.; Lenucci, M.S.; Pék, Z.; Hdider, C. Organically grown high-lycopene tomatoes: A novel adventure within functional quality. *Acta Hortic.* **2019**, *1233*, 67–72. [CrossRef]
2. Food and Agriculture Organization of the United Nations (FAO). Available online: https://www.fao.org/land-water/databases-and-software/crop-information/tomato/en/ (accessed on 13 December 2021).
3. Tomato News–WPTC Preliminary 2020 Global Crop Estimate. Available online: https://www.tomatonews.com/en/wptc-preliminary-2020-global-crop-estimate-_2_1175.html (accessed on 13 December 2021).
4. Perpar, A.; Udovč, A. Organic farming: A good production decision for slovenian small size farms and farms in the areas with restrictions/limitations or natural obstacles for agriculture. In *Multifunctionality and Impacts of Organic and Conventional Agriculture*; Moudrý, J., Mendes, K.F., Bernas, J., Teixeira, R.S., Sousa, R.N., Eds.; IntechOpen: London, UK, 2019. [CrossRef]
5. Maples, M.; Interis, M.G.; Morgan, K.L.; Harri, A. Southeastern consumers' willingness to pay for environmental production attributes of fresh tomatoes. *J. Agric. Appl. Econ.* **2018**, *50*, 27–47. [CrossRef]
6. Ventura-Lucas, M.R.; Marreiros, C. Consumer behaviour towards organic food in Portugal. In *Consumer Attitudes to Food Quality Products. EAAP–European Federation of Animal Science*; Klopčič, M., Kuipers, A., Hocquette, J.F., Eds.; Wageningen Academic Publishers: Wageningen, The Netherland, 2013; Volume 133. [CrossRef]
7. Alshaal, T.; El-Ramady, H.R. Foliar application: From plant nutrition to biofortification. *EBSS* **2017**, *1*, 71–83. [CrossRef]
8. Li, H.; Lian, C.; Zhang, Z.; Shi, X.; Zhang, Y. Agro-biofortification of iron and zinc in edible portion of crops for the global south. *Adv. Plant Agric. Res.* **2017**, *6*, 52–54. [CrossRef]
9. Abbaspour, N.; Hurrell, R.; Kelishadi, R. Review on iron and its importance for human health. *J. Res. Med. Sci.* **2014**, *19*, 164–174. [PubMed]
10. Roohani, N.; Hurrell, R.; Kelishadi, R.; Schulin, R. Zinc and its importance for human health: An integrative review. *J Res. Med. Sci.* **2013**, *18*, 144–157. [PubMed]
11. Pagani, A.; Sawyer, J.E.; Mallarino, A.P. Site-specific nutrient management: For nutrient management planning to improve crop production, environmental quality, and economic return. *Ext. Outreach Publ.* **2013**, *1*, 116.
12. Cornell University, Northeast Region Certified Crop Adviser (NRCCA). Nutrient Management. Available online: https://nrcca.cals.cornell.edu/soilFertilityCA/CA1/CA1_print.html (accessed on 14 December 2021).
13. Coelho, A.R.F.; Lidon, F.C.; Pessoa, C.C.; Marques, A.C.; Luís, I.C.; Caleiro, J.; Simões, M.; Kullberg, J.; Legoinha, P.; Brito, M.; et al. Can foliar pulverization with $CaCl_2$ and $Ca(NO_3)_2$ trigger Ca enrichment in *Solanum tuberosum* L. tubers? *Plants* **2021**, *10*, 245. [CrossRef] [PubMed]
14. Pelica, J.; Barbosa, S.; Reboredo, F.; Lidon, F.; Pessoa, M.F.; Calvão, T. The paradigm of high concentration of metals of natural or anthropogenic origin in soils–the case of Neves-Corvo mine area (southern Portugal). *J. Geochem. Explor.* **2018**, *186*, 12–23. [CrossRef]
15. Coelho, A.; Pessoa, C.; Marques, A.; Luís, I.; Daccak, D.; Silva, M.M.; Simões, M.; Reboredo, F.; Pessoa, M.; Legoinha, P.; et al. Natural mineral enrichment in *Solanum tuberosum* L. cv. Agria: Accumulation of Ca and interaction with other nutrients by XRF analysis. In *Biology and Life Sciences Forum*; MPDI: Basel, Switzerland, 2020; Volume 1. [CrossRef]
16. Doolette, C.L.; Read, T.L.; Li, C.; Scheckel, K.G.; Donner, E.; Kopittke, P.M.; Schjoerring, J.K.; Lombi, E. Foliar application of zinc sulphate and zinc EDTA to wheat leaves: Differences in mobility, distribution, and speciation. *J. Exp. Bot.* **2018**, *69*, 4469–4481. [CrossRef] [PubMed]
17. Costa, F.; Baeta, M.L.; Saraiva, D.; Veríssimo, M.T.; Ramos, F. Evolution of mineral contents in tomato fruits during the ripening process after harvest. *Food Anal. Method.* **2011**, *4*, 410–415. [CrossRef]
18. Jarquín-Enríquez, L.; Mercado-Silva, E.M.; Maldonado, J.L.; Lopez-Baltazar, J. Lycopene content and color index of tomatoes are affected by the greenhouse cover. *Sci. Hortic.* **2013**, *155*, 43–48. [CrossRef]

19. Gonzalez-Cebrino, F.; Lozano, M.; Ayuso, M.C.; Bernalte, M.J.; Vidal-Aragon, M.C.; Gonzalez-Gomez, D. Characterization of traditional tomato varieties grown in organic conditions. *Span J. Agric. Res.* **2011**, *9*, 444–452. [CrossRef]
20. Coelho, A.R.F.; Pessoa, C.C.; Marques, A.C.; Luís, I.C.; Daccak, D.; Simões, M.; Reboredo, F.H.; Pessoa, M.; Silva, M.M.; Legoinha, P.; et al. Agronomic biofortification with Fe and Zn in organic tomatoes (*Lycopersicum esculentum* L.). In Proceedings of the 1st International Conference on Water Energy Food and Sustainability (ICoWEFS 2021), Leiria, Portugal, 10–12 May 2021; Galvão, J., Brito, P., dos Santos, F., Craveiro, F., Almeida, H., Vasco, J., Neves, L., Gomes, R., Mourato, S., Ribeiro, V., Eds.; Springer Nature: Cham, Switzerland, 2021. [CrossRef]