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Micronutrient Fertiliser Reinforcement by Fulvate–Lignosulfonate Coating Improves Physiological Responses in Tomato

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Abstract: Micronutrients are essential to plants, and enhancing their availability is one of the agronomic challenges to improving crop quality and yield. This study, under controlled greenhouse conditions, compares tomato plants' responses to two different micronutrient EDTA-chelated formulations, one of them including a newly developed fulvate–lignosulfonate coating. Growth, yield, and several physiological parameters, including photosynthetic gas exchange, water-use efficiency, leaf nutrient content, leaf greenness and the effective quantum yield of photosystem II, were measured to compare their efficiency. The results showed that the new coated formulation significantly improved growth and most of the determined physiological parameters. At the end of the experiment, higher foliar levels of Fe (2.4-fold) and Mn (2.9-fold) were measured, revealing increased availability of lignofulfonate-complexed micronutrients compared to the traditional fertiliser. Moreover, the photosynthesis rate and stomatal conductance were 9- and 20-fold higher, respectively, than when using the standard fertiliser. In conclusion, the new coated fulvate–lignosulfonated fertiliser provided a more suitable source of micronutrients for tomato plant fertilisation, allowing for higher yields, which correlated with a generally improved physiological response.

Keywords: chelate fertiliser; humic acid; lignosulfonate; natural polymers; micronutrient uptake; *Solanum lycopersicum*

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1. Introduction

The tomato (*Solanum lycopersicum*) is globally the second most important horticultural crop after the potato, partly due to its many beneficial health properties [1,2]. Its high content of antioxidants, mainly in the form of carotenoids, confers tomato fruits a relevant role in the human diet to preserve health [3]. According to FAOSTAT [4], the world production of tomatoes in 2021 was ca. 189 million tonnes in a total harvested area of around 5.2 million ha. In Mediterranean countries, tomato consumption is approximately 10 kg per person per year, although it is notably higher in countries like Spain and Italy [5]. Based on data from the Spanish Chamber of Commerce [6], exports of fresh or chilled tomatoes from Spain exceeded 0.7 million tonnes in 2021, valued at about 10^9 Euros.

Micronutrients are essential for humans to avoid nutritional deficiencies, so improving crop yields and harvest nutritional quality is crucial for a healthy diet [7]. According to Katyal and Randhawa [8], some aspects, like intensive cropping, use of wide-yielding varieties, loss of micronutrients through leaching and liming, or an increasing proportion

of chemical fertilisers compared to organic fertilisers, are making soils poorer. Several reviews explain the importance of micronutrients as they are essential substances that plants use [9–12]. They play critical catalytic roles in the metabolism of plants by acting as multiple enzyme cofactors in many physiological functions, including photosynthesis, stomatal regulation, osmoregulation, disease resistance and cell wall formation [13].

Soil's physical properties naturally improve with average-to-high organic matter levels [14]. Ecological intensification in agriculture means that the use of humic substance (HS)-based products has significantly increased over the last years [15–17]. Huge efforts have been made to know the structure and composition of HSs [18]. Classically, different fractions can be separated according to their solubility in acid/alkaline solutions [19,20]. In this way, a colloidal dispersion is formed with dark-brown hydrophobic precipitates, namely humic acids (HAs), and a yellow hydrophilic supernatant fraction, namely fulvic acids (FAs). It has been demonstrated that the application of HSs improves soil physicochemical and microbiological properties by increasing fertiliser efficiency and improving plant metabolism [21–23]. Low-molecular-weight humic compounds can be absorbed by roots and translocated to shoots, where they enhance different metabolic processes [24]. The complexing properties of HSs allow for an increase in cation exchange to occur [25]. However, the benefits depend largely on the HSs' organic nature, industrial processing, concentrations, soil properties, and culture methods [19]. In fact, a lack of efficiency due to different aspects, like rapid lixiviation [26] or overdosing, has sometimes been reported [27].

Lignosulfonates (LSs) are water-soluble anionic polyelectrolyte lignin polymers produced during the wood-pulping process by the paper industry or extracted from coal mines [28–30]. LSs share properties with HSs in chelation, buffering and cation exchange capacity [31], offering excellent proven benefits for plants as they enhance plant growth and disease management [32]. Their use in agriculture as natural complexing agents in fertilisers, formulated with macro- and micronutrients, is relatively recent [33]. Some research works have highlighted the positive effects of LSs on nutrient availability for plants by applying different fertiliser formulations, including ammonium, urea, Ca and K [28,32,34,35]. These natural polymers are also used as chelating agents for micronutrients like Fe, Mn and Zn [23,36–38]. Compared to synthetic chelates, LSs are low-cost, biodegradable and environmentally friendly.

The novelty of this study lies in the development of a micronutrient formulation, including lignosulfonates as natural complexing agents together with fulvates (humic substances) acting as biostimulants, which should promote microbial activity and favour plant nutrient availability. Until now, research on HSs has focused mainly on treatments applying barely processed organic amendments or watering/spraying liquid fertilisers. Despite their proven beneficial effects on micronutrient availability, few studies have been carried out with LS–HS products and focused mainly on laboratory conditions or different crops like cereals [31,39,40]. This study, performed for the first time in tomatoes, aimed to compare the effects of a fulvate-LS-coated micronutrient fertiliser to those of a traditional one of a similar composition by determining differences in plant growth, nutrient uptake and photosynthetic responses to validate their enhanced efficiency.

2. Materials and Methods

2.1. Experimental Design and Fertiliser Treatments

The fresh-marketed tomato cultivar H-9776 (Heinz Ibérica, La Rioja, Spain) was chosen to undergo the experiment based on its adequacy for greenhouse cultivation. Seeds were sown individually in pots (diam. of 13.2 cm, depth of 14.2 cm) with holes at the bottom and filled with poor clay-loam soil. The pots were, in turn, placed in polyethylene trays (dimensions: 60 × 40 × 7 cm) filled with white peat (pH 5.5–6.5) to favour root growth. Each treatment consisted of 24 plants (i.e., 24 pots placed in 4 trays, 6 pots per tray). Seedlings were grown for 175 days in a greenhouse in the Valencian Institute of Agricultural Research (IVIA) facilities (Moncada, Spain) under the following ambient conditions: 19–27 °C, 60–80% relative humidity and natural daylight.

Two kinds of micronutrient fertilisers were tested at 0.33 kg m^3 by application (i.e., a litre of solution was distributed uniformly between 6 pots per tray)—for greenhouse horticultural cultivations, a dose of 2–5 $\times$ 10^{-4} kg m^{-2} applied 2 to 5 times in the irrigation water is recommended: (1) Mix Nutricionales Agrofit® (Agrofit S. Coop., Picassent, Valencia, Spain), product barcode 8 43600205 0187, with the following composition (%, *w/w*): Fe–EDTA (7.5), Mn–EDTA (3.3), Zn–EDTA (0.6), Cu–EDTA (0.3), B (0.7), Mo (0.1), considered as 'traditional' (Treatment A—henceforth abbreviated as "TA"); and (2) Microquel Mix® (Fertinagro Biotech S.L., Teruel, Spain), a fulvate–lignosulfonate (FA–LS)-coated micronutrient fertiliser, with the following declared composition (%, *w/w*): Fe–EDTA (4.5), Fe–LS (3), Mn–EDTA (2), Mn–LS (1.3), Zn–EDTA (0.6), Cu–EDTA (0.5), B (0.7), Mo (0.2), EDTA chelating agent (30%) and complexing agent (lignosulfonic acid) (Treatment B—"TB"). The total dose of the different micronutrient formulations, applied per plant during the whole experimental period, was 0.33 g and distributed as follows: Fe (0.02475 g), Mn (0.01089 g), Zn (0.00198 g), Cu (0.00099 g in TA, and 0.00165 g in TB), B (0.00231 g) and Mo (0.00033 g in TA, and 0.00066 g in TB) (equivalent to the maximum recommended dose of 5 $\times$ 10^{-4} kg m^{-2} for a planting frame of 0.45 $\times$ 0.3 m^2). Both micronutrient fertilisers were combined with an inorganic fertiliser NPK 15:15:15 (i.e., composed of 15% nitrogen, 15% phosphorous and 15% potassium) (BASF Coating Services, Ludwigshafen. Germany) applied at a dose of 2 kg m^3 (equivalent to applying a total amount of 0.95 g of N, P and K per plant, in a similar manner as for the micronutrients) according to the schedule summarised in Table 1. Control plants did not receive any micronutrient fertiliser treatment.

Table 1. Schedule of fertilisation days for treatments A and B from sowing to the experiment's conclusion at 175 days. Applied treatments: NPK (15N–15P–15K) complex fertiliser applied at a dose of 2 kg m^3; NPK + micronutrient fertilisers applied at a dose of 0.33 kg m^3.

Phenological Stage	Days from Sowing	Applied Treatments
Vegetative growth	49, 54, 57, 61, 65, 70, 73, 76, 78	NPK
Flowering	82, 86, 90, 103, 109	NPK + Micronutrients
	133, 135, 143	NPK
Fruit setting	151	NPK + Micronutrients
	165	NPK

2.2. Soil Fertility Characterisation

Several soil characteristics were examined to determine soil fertility. The pH and EC of a 1/5 (*w/v*) aqueous soil extract were assessed by shaking for 2 h, centrifugation at 27,000$\times$ *g* for 15 min, and filtering. A pH meter (Crison mod. 2001, Barcelona, Spain) was used to measure pH, and a conductivity meter (Crison micro CM2200, Barcelona, Spain) was used to measure electrical conductivity (EC). After removing carbonate by acid digestion with HCl, total and organic soil carbon and total nitrogen (N) were determined using combustion gas chromatography in a Flash EA 1112 Thermo Finnigan (Franklin, MA, USA) elemental analyser. The total nutrient contents (P, K, Ca, Mg, Cu, Fe, K, Mg, Mn and Zn) were extracted and determined using aqua regia digestion (3:1, *v/v*, HCl/HNO_3) and determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP–AES) (Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA). According to the soil analyses, the experiment was conducted on nutrient-poor soil (Table 2).

Table 2. Chemical properties of experimental soil. Data on total nitrogen, total carbon and organic carbon, pH, electrical conductivity (EC), and macro- and micronutrient contents from the top 15 cm of soil surface are shown. Values are means ± SE (n = 5) at the beginning of the experiment.

Parameters	Mean ± SE
Total nitrogen (g 100 g^{-1})	0.09 ± 0.01
Total carbon (g 100 g^{-1})	2.06 ± 0.13
Organic carbon (g 100 g^{-1})	0.66 ± 0.03
pH	8.75 ± 0.04
EC (µS cm^{-1})	120.7 ± 12.52
P (g 100 g^{-1})	0.06 ± 0.0004
K (g 100 g^{-1})	0.34 ± 0.06
Mg (g 100 g^{-1})	0.29 ± 0.02
Ca (g 100 g^{-1})	3.56 ± 0.19
Fe (g 100 g^{-1})	11.11 ± 0.79
Cu (mg kg^{-1})	15.93 ± 0.65
Mn (mg kg^{-1})	191.99 ± 5.22
Zn (mg kg^{-1})	26.95 ± 1.12

2.3. Growth Analysis

Six tomato plants per treatment were selected to study plant growth, development and fruit production at the end of the experiment when plants were harvested (i.e., on day 175 after sowing) to determine the following parameters: primary stem length, total stem length (including the main stem and secondary branches), stem diameter, leaf number, leaf weight, foliar area, total fresh weight of the aerial part, dry weight of the aerial part, root fresh mass, root dry mass, flower number, fruit number and fruit mass. The stem diameter was measured using a digital standard gage vernier calliper (PCE inst., Albacete, Spain). Six plants per treatment were randomly selected, and a minimum of two leaves per plant were scanned to estimate the foliar area using Image J 1.47 Software (NIH, Bethesda, MD, USA). The selected leaves were the "most recently mature leaves" (MRML) [41], typically the fourth or fifth leaves down from the growing point. A fraction of the plants' aerial and root parts was weighed fresh weight (FW), dried for 72 h at 65 °C and weighed again dry weight (DW) to calculate the percentage of the corresponding dry mass. The remaining plant material was frozen and stored at –20 °C for further analyses. Fruit mass was calculated from the average of a minimum of 10 mature fruits per plant (6 plants per treatment).

2.4. Gas Exchange Analysis

For each treatment (six plants per treatment), 10 MRML leaves were selected and labelled to measure gas exchange using a portable infrared gas analyser Lcpro–SD and by incorporating a PLU5 LED light unit (ADC BioScientific Ltd., Hoddesdon, UK). Measurements were taken at noon (11–15 h) on days 169, 170 and 173 (clear days without clouds so that the plants received the highest light intensity) when plants were mature and producing fruits. The studied parameters were the stomatal conductance (*gs*) (expressed as mmol m^{-2} s^{-1}), the net photosynthetic rate (*A*) (µmol m^{-2} s^{-1}), transpiration (*E*) (mol m^{-2} s^{-1}), and the intercellular CO_2 concentration (*Ci*) (µmol mol^{-1}), measured under ambient CO_2, temperature, and relative humidity conditions. They were recorded using photosynthetically active radiations (PAR) ranging from 400 to 1800 µmol m^{-2} s^{-1} to determine the light saturation point. Water-use efficiency (WUE) and intrinsic WUE were calculated as the ratio between *A/gs* and *A/E*, respectively, expressed as µmol (CO_2 assimilated) mol^{-1} (H_2O transpired).

2.5. Leaf Greenness and Effective Quantum Yield of Photosystem II

Greenness was measured by a SPAD-502 Chlorophyll meter (Konica-Minolta, Osaka, Japan). The effective quantum yield of photosystem II electron transport (*ΦPSII*), represent-

ing the electron transport efficiency between photosystems within light-adapted leaves, was checked daily using a leaf fluorometer (Fluorpen FP100, Photos System Instrument, Drasov, Czech Republic). Each measurement (*ΦPSII* and greenness) was taken on one or two mature leaves per plant, from all treated plants (24), on days 169 and 173, at noon.

2.6. Leaf Nutrient Contents

Nutrient content was determined in the MRML leaves previously collected on day 175 and kept at –20 °C. The macro- (N, P, K, Ca, Mg, S) and micro- (Fe, Cu, Mn, Zn, B and Mo) nutrient contents were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES); each composite sample consisted of 10 MRML leaves per plant, and six plants per treatment were analysed. The N content was also estimated by the handheld reflectance meter N–Pen N 100 apparatus (Photon System Instruments, Drásov, Czech Republic), with one measurement per plant from 12 plants per treatment on days 169 and 173.

2.7. Statistical Analysis

The data collected from the vegetative, reproductive and photosynthetic parameters were compared between treatments and the control by analysis of variance (one-way ANOVA) at the 95% confidence level using "treatment" as the grouping factor. Prior to the use of ANOVA, the normality and homogeneity requirements of variances were checked according to Levene's and Shapiro–Wilk's tests. When the null hypothesis was rejected, post hoc comparisons were made to establish the possible statistical differences between the different applied treatments by Tukey's test. Principal Component Analysis (PCA), using Pearson's correlation coefficients, was applied for multiple sample comparisons of physiological variables and treatments. For this analysis, data of the photosynthetic parameters (*gs*, *E*, *A*, *Ci*, *A/gs* and *A/E*) are those obtained at 1000 µmol m^{-2} s^{-1} PAR at the end of culture, on days 169, 170 and 173. Data are shown as means and standard errors. Statistical Statgraphics Centurion v.15 (Statgraphics Technologies, Inc., The Plains, VA, USA) software was used to perform the analyses.

3. Results

3.1. Impact of Different Fertilisers on Plant Growth and Development

At the end of the experiment, the application of both fertiliser treatments significantly affected all quantified parameters related to vegetative and reproductive growth compared to the controls (Figure 1, Table 3).

Table 3. Effects of control and fertilizer A and B treatments on vegetative growth and the development of reproductive organs in tomato plants. Values are means ± SE (n = 6) at the end of the experiment, on day 175 after sowing.

Parameter	Control	Treatment A	Treatment B
Primary stem length (cm)	78.75 ± 3.21a	132.83 ± 11.33b	144.67 ± 4.75b
Total stem length (cm)	78.75 ± 3.21a	205.17 ± 37.45b	292.08 ± 80.59c
Stem diameter (mm)	5.48 ± 0.21a	7.90 ± 0.48b	8.25 ± 0.72b
Leaf number	13.83 ± 1.17a	42.50 ± 4.15b	65.33 ± 9.94c
Leaf weight (g)	2.71 ± 0.53a	9.21 ± 0.97b	9.03 ± 0.72b
Foliar area (mm^2)	104.57 ± 13.36a	304.59 ± 34.49b	274.63 ± 27.65b
Total fresh weight (aerial part) (g)	49.10 ± 5.04a	298.31 ± 35.83b	424.30 ± 65.61b
Dry weight (aerial part) (g)	19.79 ± 2.33b	59.97 ± 7.12a	74.37 ± 13.64a
Root fresh weight (g)	5.20 ± 2.53a	48.67 ± 12.51b	83.88 ± 20.98b
Root dry weight (g)	2.80 ± 1.34b	16.28 ± 4.25a	28.02 ± 8.07a
Flower number	8.50 ± 2.49a	6.00 ± 1.77a	61.00 ± 18.95b
Fruit number	0	6.50 ± 1.59a	9.67 ± 5.02a
Fruit weight (g)	–	39.68 ± 8.10b	40.36 ± 8.24b

Different letters in the same row indicate statistically significant differences between treatments (Tukey test, $p \leq 0.05$).

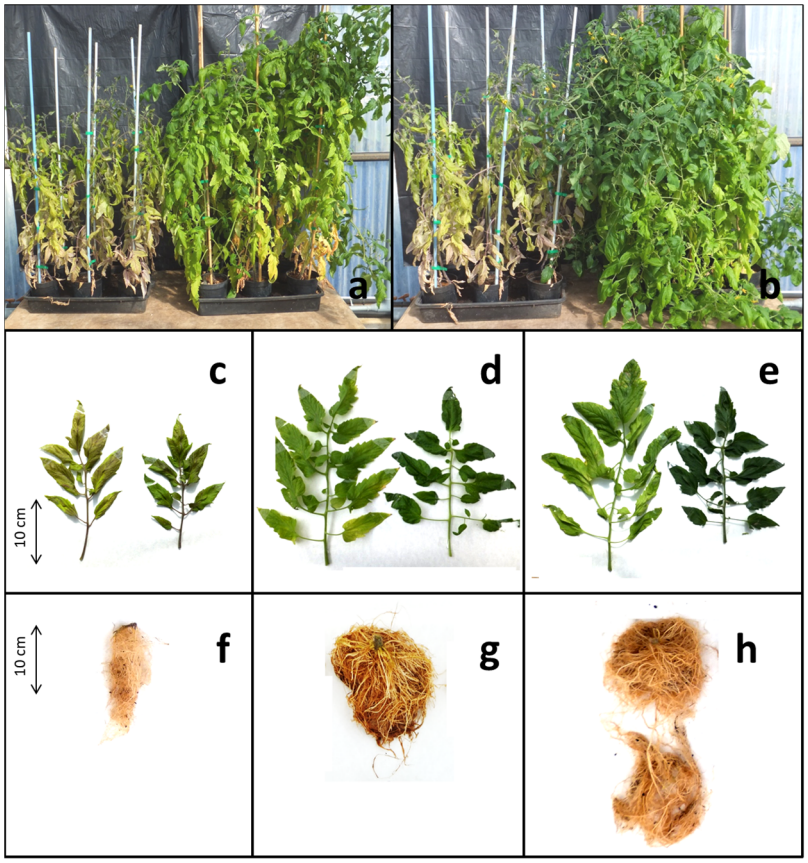

Figure 1. Effects of fertiliser treatments on vegetative development (**a**,**b**), foliar area (**c**–**e**) and maximum root development (**f**–**h**) in tomato plants at the end of the experiment, on day 175 after sowing. (**a**): Treatment A (right) compared to the control (left); (**b**): treatment B (right) compared to the control (left); (**c**,**f**): control; (**d**,**g**): treatment A; (**e**,**h**): treatment B. Arrows correspond to 10 cm (**c**–**h**).

The total stem length (1.4-fold) and leaf number (1.5-fold) of plants were significantly higher in TB than in TA plants. On average, TB's primary stem length and diameter were slightly higher. Leaf weight and foliar area were practically the same in both treatments. The total fresh weight of the aerial part and the root were, on average, 1.4- and 1.9-fold higher in TB than in TA. The dry matter percentages of the aerial part and the root were, on average, higher in TA than in TB, in accordance with the higher turgor levels observed in TB (Table 3). These results agree with the differences in vegetative growth observed visually between control plants and those grown with micronutrient fertilisers (treatments A and B) (Figure 1).

Although yields were lower than expected, due mainly to very low temperatures during the fruit set, higher production was observed in TB than in TA. Control plants did not bear fruit at the end of the experiment (i.e., 175 days after sowing), whereas fruits of TA and TB plants were not mature but collected at the phenological state of the fruit formation. On average, flower and fruit numbers were 10.2- and 1.5-fold higher in TB than TA, respectively, but significant differences were detected only for the flower number and not in the fruit number and fruit weight (Table 3).

3.2. Gas Exchange, Leaf Greenness and ΦPSII

Results on gas exchange parameters (*gs*, *E*, *A* and *Ci*) measured on days 169, 170 and 173 after sowing are shown in Figure 2. *A* and *gs* were initially measured in the 40-day-old plants for TA, TB and control treatments under a 1000 µmol m^{-2} s^{-1} PAR, as the light saturation point was observed to be produced around this PAR. However, no significant differences were observed between the treatments, and the *gs* and *A* values were

about 0.12 µmol m^{-2} s^{-1} and 10 µmol m^{-2} s^{-1}, respectively. On days 169, 170 and 173, the *gs* (Figure 2a), *A* (Figure 2b), transpiration rate (*E*, Figure 2c) and substomatal CO_2 concentration (*Ci*, Figure 2d) parameters were measured with PAR values ranging from 400 to 1800 µmol m^{-2} s^{-1}. The light saturation point was found to match the results with the 40-day-old TB plants mentioned above, also occurring at 1000 mol m^{-2} s^{-1} PAR. For the first three variables, no significant differences were found between all the measurements within the tested range of PAR values for each treatment; in all cases, MRML leaves taken from control and TA adult plants showed similar *gs*, *A* and *E* values, which were substantially lower than those of TB–MRML. For example, stomatal conductance in the leaves of TB plants (ca. 150 mmol m^{-2} s^{-1}) was about 10- to 20-fold higher than in the control and TA plants (Figure 2a). Similarly, *A* in TB plants (about 10 µmol m^{-2} s^{-1}, on average) was ca. 9-fold higher than that in TA and control plants (Figure 2b), and *E* (about 3 mol m^{-2} s^{-1}) was ca. 6- to 15-fold higher than those in the control and TA plants, respectively (Figure 2c). No clear pattern of *Ci* in the different treatments was observed at low PAR values, whereas at PAR $\geq$ 1000 µmol m^{-2} s^{-1}, CO_2 levels were consistently the highest in control plants and lowest in TA, with intermediate values measured in TB plants (Figure 2d).

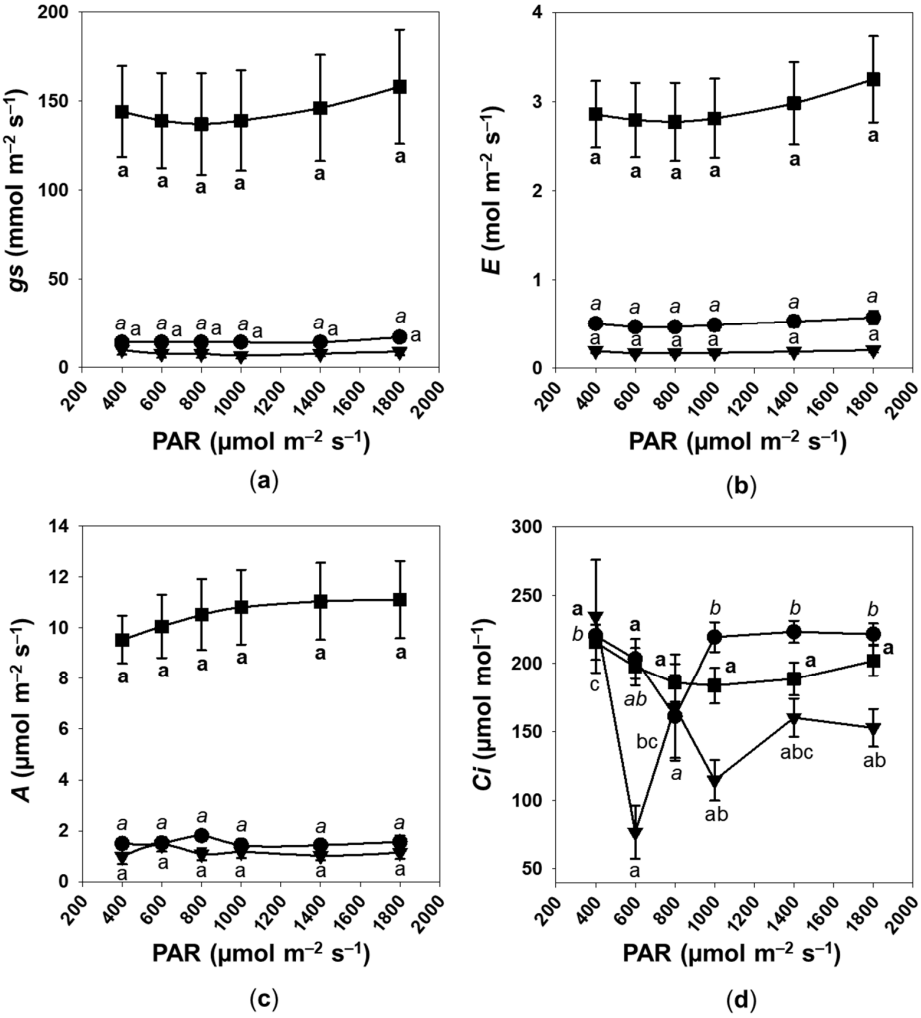

Figure 2. Gas exchange responses to the different photosynthetically active radiation rates of tomato plants treated with fertilizers A (▼), B (■) and the control (●). (**a**) Stomatal conductance—*gs*, (**b**) transpiration rate—*E*, (**c**) net photosynthetic rate—*A* and (**d**) substomatal CO_2 concentration—*Ci*. Values represent the mean ± SE (n = 10) at the end of the experiment, of measurements taken on days 169, 170 and 173 after sowing. Different letters for each treatment indicate significant statistical differences (Tukey test, $p \leq 0.05$).

Regarding WUE and intrinsic WUE, on day 173, the *A/gs* (Figure 3a) and *A/E* (Figure 3b) ratios generally showed higher values for TA compared to TB and the control. Maximum values of WUE and intrinsic WUE were recorded at 600 and 1000 PAR for TA plants (ca. 160 µmol mol^{-1}), significantly higher than in TB (ca. 1.5-fold) and control (ca. 2.5-fold) plants (Figure 3). Higher TA levels were not associated with higher *A* levels. For all investigated PAR, TB showed more consistent WUE values, indicating effective photosynthetic responses. Given that in both treatments, the plants received the same irrigation, differences between TB and TA could not be attributed to the set of plants subjected to one of the treatments having the ability to withstand drought better than the other.

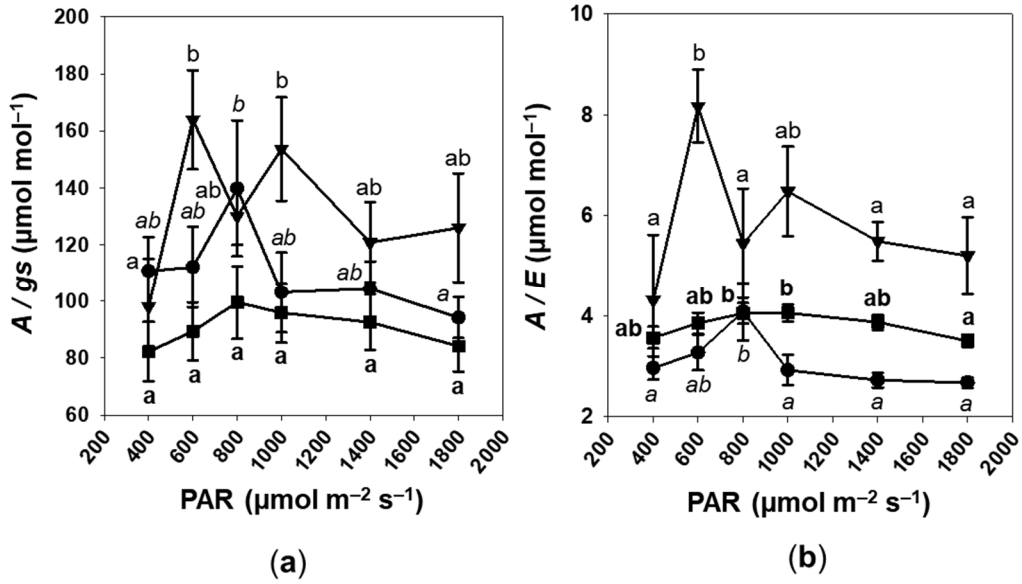

Figure 3. Water-use-efficiency responses to the different photosynthetically active radiation rates of tomato plants treated with fertilizers A (▼), B (■) and control (●). (**a**) Water-use efficiency—*A/gs* and (**b**) intrinsic water-use efficiency—*A/E*. Values represent the mean ± SE (n = 10) at the end of the experiment, with measurements taken on days 169, 170 and 173 after sowing. Different letters for each treatment indicate significant statistical differences (Tukey test, $p \leq 0.05$).

Results on *ΦPSII*, leaf greenness content and N content measured by reflectance are shown in Table 4. The *ΦPSII* of the TB plants was significantly higher (ca. 1.3-fold) than that of the TA and the control plants. The leaf greenness of the TB plants, measured as SPAD units, also showed a significantly higher value than that of the TA and the control plants, ca. 1.3- and 2.4-fold higher, respectively. The N content increased significantly in both micronutrient fertiliser treatments with respect to the control plants, also showing a significantly higher value in TB plants compared to treatment A (Table 4).

Table 4. Effects of fertiliser treatments on the effective quantum yield of photosystem II (*ΦPSII*), leaf greenness and nitrogen content measured by reflectance in tomato plants. The values are the means ± SE (n = 6) at the end of the experiment on days 169 and 173 after sowing. N content estimated by the handheld reflectance meter (N Pen N 100).

Parameters	Age (days)	Control	Treatment A	Treatment B
ΦPSII	169	0.54 ± 0.02a	0.57 ± 0.03a	0.69 ± 0.01b
	173	0.56 ± 0.03a	0.54 ± 0.03a	0.73 ± 0.01b
Leaf greenness (SPAD units)	169	14.62 ± 1.44a	28.59 ± 2.03b	36.24 ± 1.47c
	173	15.57 ± 1.55a	27.00 ± 1.36b	38.26 ± 1.51c
N content (%)	169	2.07 ± 0.84a	2.30 ± 0.94b	2.86 ± 1.17c
	173	2.05 ± 0.84a	2.20 ± 0.9b	2.89 ± 1.18c

Different letters in the same row indicate statistically significant differences between treatments (Tukey test, $p \leq 0.05$).

3.3. Foliar Content of Macro- and Micronutrients

The macro- and micronutrient contents measured in leaves are presented in Table 5. The foliar content in the control plants was significantly lower for N, P, K and Zn compared to both treatments with micronutrients, TA and TB. Significant differences were observed in control versus TB but not versus TA plants for Mg, Fe, Cu, Mn, Mo and B. No differences were found between the treatments and the control for Ca. Regarding the differences between both applied treatments, P, Fe, Cu, Mn and Mo were significantly higher in TB than in TA. The extra Cu (0.2%) and Mo (0.1%) added in treatment B produced ca. 1.6-fold and 2-fold higher assimilation of these micronutrients, respectively, in leaves of TB than of TA plants. Also, increased levels of Fe (2.4-fold) and Mn (2.9-fold) were observed in TB compared to TA, agree with an improved efficiency by adding Fe–LS and Mn–LS in TB. The macronutrient contents measured in the control plants did not reach the sufficiency concentrations, except for Ca. On the contrary, all micronutrients in control plants were included within the sufficiency range, although at low levels except for Mo (Table 5).

Table 5. Effects of fertiliser treatments on the foliar content of macro- (N, P, K, Ca, Mg) and micronutrients (Fe, Cu, Mn, Zn, Mo and B) in tomato plants, measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP–OES). Values are the means ± SE (n = 6) expressed on a dry weight basis at the end of the experiment (on day 175 after sowing).

Parameter	Sufficiency *	Control	Treatment A	Treatment B
N (%)	3.5–5	0.82 ± 0.16a	4.38 ± 0.52b	4.18 ± 0.57b
P (%)	0.3–0.65	0.11 ± 0.02a	0.39 ± 0.1b	0.65 ± 0.18c
K (%)	3.5–4.5	3.01 ± 1.08a	5.52 ± 1.28b	6.02 ± 1.1b
Ca (%)	1–3	2 ± 0.25a	2.1 ± 0.7a	2.4 ± 1.17a
Mg (%)	0.35–1	0.32 ± 0.09a	0.51 ± 0.11ab	0.62 ± 0.14b
Fe (ppm)	50–300	92.25 ± 12.66a	103 ± 19.37a	252 ± 149.36b
Cu (ppm)	5–35	6.5 ± 1.29a	9.75 ± 2.63a	15.2 ± 4.82b
Mn (ppm)	25–200	34 ± 7.26a	38.75 ± 11a	110.6 ± 52.61b
Zn (ppm)	18–80	34.5 ± 3.7a	38.21 ± 6.8b	42.8 ± 14.1b
Mo (ppm)	0.1–1	0.78 ± 0.26a	1.5 ± 0.4a	2.96 ± 0.72b
B (ppm)	30–75	42.25 ± 14.8a	56.25 ± 14.86ab	86.6 ± 34.93b

Different letters in the same row indicate statistically significant differences between treatments (Tukey test, $p \leq 0.05$). B, Fe, Mo and N were transformed as 1/(1–LOG(X)) to be adapted to a normal distribution. * Sufficiency ranges [42].

3.4. Principal Component Analysis (PCA)

To analyse the correlations between physiological responses and micronutrient treatments, a PCA including all photosynthetic parameters (*gs*, *A*, *E*, *Ci*, *A/gs* and *A/E*), *ΦPSII*, leaf greenness, N content determined by reflectance, macro (N, P, K, Ca and Mg) and micronutrients (Fe, Cu, Mn, Zn, B and Mo) and factorial inertia of micronutrient treatments was performed using Pearson's correlation method (Figure 4).

The biplot (F1, F2) explained 67.32% of the variance in the examined parameters. The physiological factors with the highest inertia on the F1 component (explaining 52.46% of the variability) were *A*, *gs*, *E*, *ΦPSII*, leaf greenness, N content by reflectance, and all macro- and micronutrients assessed, except Ca, Na and Zn. *A* was positively correlated with *gs* (r = 0.98), *E* (r = 0.987), *ΦPSII* (r = 0.714), leaf greenness (r = 0.668), N content by reflectance (r = 0.844), N (r = 0.553), P (r = 0.863), K (r = 0.494), Fe (r = 0.569), Cu (r = 0.623), Mn (r = 0.683), Zn (r = 0.438) and B (r = 0.827), and negatively correlated with *A/gs* and Mo, according to Pearson's correlations. Major inertia of parameters associated with physiological response improvement occurred in relation to TB, whereas TA and the control were mainly placed on the negative F1 axis or close to zero. The F2 axis accounted for only 14.86% of the data variability, with *Ci* (positively) and *A/gs* and *A/E* (negatively) presenting significant inertia on this axis. Most photosynthetic and nutritional parameters were closely correlated to TB, supporting the previous results showing a better physiological response for TB than for TA and the control.

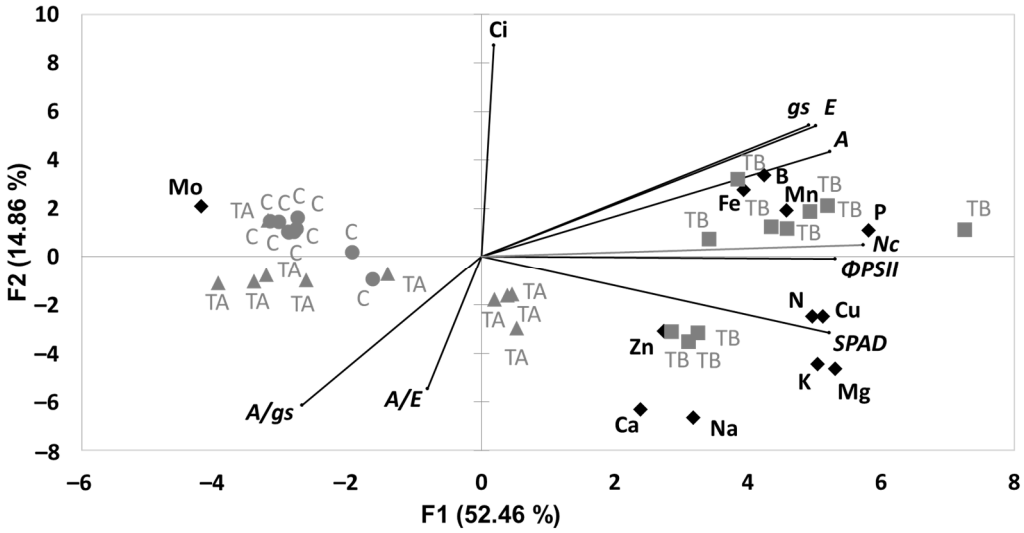

Figure 4. Principal Component Analysis (PCA). Biplot correlations between physiological parameters and micronutrient treatments. Photosynthetic parameters were measured at 1000 μmol m^{-2} s^{-1} PAR on days 169, 170 and 173 after sowing (stomatal conductance—*gs*, net photosynthetic rate—*A*, transpiration—*E*, intercellular CO_2 concentration—*Ci*, water-use efficiency—*A/gs*, and intrinsic water-use efficiency—*A/E*); and on days 169 and 173, the effective quantum yield of photosystem II electron transport (*ΦPSII*), leaf greenness (SPAD units) and N content, whose F1 and F2 axis inertia are indicated in black lines, were measured by reflectance. Macro- (N, P, K, Ca and Mg) and micronutrient (Fe, Cu, Mn, Zn, B and Mo) correlations are represented by diamonds. Each triangle (TA), square (TB) and circle (control) represents the correlation output for each micronutrient treatment and control. Correlations were performed by Pearson's method ($p \leq 0.05$).

4. Discussion

Micronutrients are essential in plant metabolism, and their positive effects on plant development are well known [10]. Micronutrient fertilisers are commonly chelated formulae, considered the most efficient way to favour plant micronutrient absorption [43]. Despite their general beneficial effects, fertilisers are sometimes not efficient enough to correct nutritional deficiencies and are generally considered environmentally unsafe [44]. The efficiency of micronutrient fertilisers strongly depends on the form they are applied (e.g., foliar application, with the irrigation water or applied to soil), which also determine nutrient availability for plants. Due to their fast decomposition, traditional fertilisers are lost to a great extent by leaching. Calcareous soils, typical in SE Spain, usually have a high pH, little organic matter and are frequently deficient in micronutrients [45]. Calcium competes with micronutrients (mainly Fe, Cu, Mn and Zn) for active soil sites, reducing their plant availability. Moreover, nutritional deficiency frequently appears in some peat soils due to their extreme acidity and low fertility [46]. In this context, the presence on the market of liquid or granular fertilisers enriched with organic matter and biostimulants is becoming increasingly common [47] since the beneficial effects of organic fertilisers are well recognised [22,48,49]. Regarding roots, fertilisers with HSs enhance the root surface by increasing the length and growth of root hairs, an activity that favours beneficial microorganisms and increases the movement of metal ions [50–52]. FA seem to affect root growth more strongly than HA [53]. One explanation is based on the fertiliser composition, as FA particles have a lower molecular weight and are slightly less aromatic and richer in carboxyl (–CO_2H) groups and carbohydrates than HA [54]. HSs also have auxin-like activity [15], activate different enzymes [55] and lower the incidence of diseases and pests [56,57]. In tomato plants, for example, applying K-humate and K-fulvate to soil and leaves increased the fresh and dry biomass of shoots and fruits and the uptake of nutrients [58]. Yields also increased in tomato plants grown under greenhouse conditions by ca. 40% by foliar applications of a liquid fertiliser containing chelated micronutrients and K-humate, com-

pared to the control [59]. Soil treatments by injecting Ca-, B-humate and humic acid into the root zone positively affected total marketable yields [60]. The combined effect of humic substances and amino acids enhanced iron availability (Fe-EDDHA) in tomato [61]. In this work, when comparing plant growth between both micronutrient treatments, fertilisation with formulation B (supplemented with FA) produced bigger plants with enhanced stem length, leaf number, water content, flower number and fruit number. Suh et al. [27] also reported increased vegetative parameters, such as plant height, fresh and dry weight, and fruit number after spraying FA onto tomato leaves. We observed that photosynthetic parameters, such as *A*, *E*, *gs*, *ΦPSII* and chlorophyll content, showed substantially higher values in plants treated with fertiliser B compared to fertiliser A and the control. The low values obtained at the end of the experiments for these two latter treatments suggested that plants had already undergone senescence induction, whereas fertiliser B allowed the growth to be maintained longer. These results partly agree with Haghighi and Teixeira Da Silva [62], who also obtained higher *A* values after applying HA. However, these authors did not analyse the effects of HA on *E* or *gs*. In some cases, no effects caused by HS were observed, and some reports have questioned their use because they did not produce any significant results [63,64]. In any case, the effects of HS on plants depend on different factors, including origin, concentration and culture method [65,66].

Although tomato plants are biologically susceptible to Fe and Cu deficiencies, the plants in both applied treatments presented levels of macro- and micronutrients that fell within normal limits [10]. A higher proportion of Cu (0.2%) and Mo (0.1%) present in formulation B did not explain the differences noted in the plants' physiological responses. However, significantly increased levels were reported in leaves for TB. It seems that Cu and Mo exerted no synergistic effects on other micronutrients, but they could have had a slight effect by increasing P levels (0.65%). It has been reported that antagonisms like excess P could negatively affect the available B, Cu and Zn contents [67,68]. In any case, although each company establishes its industrial manufacturing processes, the different responses observed between formulations A and B should be attributed to the beneficial properties of the HS–LS coating present in B. In fact, LS are complexing agents favouring micronutrient absorption by plant roots. The LS composition includes different hydroxy phenolic, carboxylic and sulfonic functional groups that can bind to macro- and micronutrients and reduce sugars [29,69]. Also, humic substances have chelating and biostimulant properties contributing to increased plant micronutrient levels [70,71]. Our results showed significantly higher levels of Fe and Mn contents in the TB plants than in those subjected to treatment A (i.e., those micronutrients that were also complexed with LS). In fact, LS has been shown in different studies to increase the availability of micronutrients. To give some examples, it has been reported that the reducing sugars present in LS can favour Fe^{2+} availability [36]. Also, higher Mn concentrations have been found in wheat shoots after applying Mn-LS [37]. As shown by some other reports, LS increased grain yields by enhancing N uptake from urea in maize [72], and Zn–LS applications increased Zn concentrations and dry weight in shoots [38,73]. Good results have been obtained in soil containing Zn when applying Zn–LS and EDTA [39,74], whereas LS increased P availability under gibbsite-rich acidic soil conditions [75]. LS and HS acted as natural complexing agents and displayed a similar functionality to natural chelates. LS are more enviro-friendly and cheaper than synthetic chelates and allow for the slow release of nutrients by the lignin polymer. As shown in this work, micronutrients coated with LS–fulvate enhanced the chelation power and nutrient availability for tomato plants.

5. Conclusions

It is shown here that lignosulfonates mixed with fulvates are useful in enhancing growth, yield, and physiological responses in tomato plants cultured in soil in the greenhouse. FA–LS-coated fertiliser (B) was more efficient than the traditional fertiliser (A), this last only EDTA-chelated. Applications of FA–LS-coated micronutrients allowed for high values for the most relevant growth parameters analysed here since photosynthetic and

nutritional parameters were favoured. This is related to superior fulvate and lignin polymer performance included in coatings due to the gradual release of micronutrients, which leads to a more extended nutrient availability. This coating also has other advantages compared to synthetic chelates since they are natural products that can be obtained abundantly and cheaply. Their complexing and chelating properties limit leaching and immobilisation of micronutrients, increasing their availability to plants. Also, their beneficial effects on soil microorganisms make these products attractive to reinforce plant growth. Because of their complexity and novelty, more research is needed to better understand their effects on different crops and agronomic conditions. Companies manufacturing and commercialising biostimulants, controlled-release fertilisers and ecological products, and the farmers themselves must be aware of the use of these natural substances in the physiological improvement of crops. Finally, the work reported here shows that this FA–LS-coated fertiliser can help obtain better yields in horticultural plants like tomatoes.

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