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Biostimulant Effects of Micro Carbon Technology (MCT®)-Based Fertilizers on Soil and *Capsicum annuum* Culture in Growth Chamber and Field

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Abstract: Due to the environmental issues that conventional fertilization is causing, biostimulants are proposed as environmentally friendly alternative for crop nutrition in agriculture. The aim of this study was to determine the effects of new Micro Carbon Technology (MCT®) fertilizers with biostimulant activity based on humic acids biologically digested from leonardite on pepper plant growth in three different soils with different textures. The assays were performed under controlled conditions in a growth chamber and in commercial greenhouses in Spain. The effects on soil were analyzed after the addition of the fertilizers by microbial respiration and enzymatic activities (hydrolase, dehydrogenase and urease). For the plant assays, biometric parameters (fresh weight and fruit hardness) and foliar analysis (chlorophyll indices and nutrients) were evaluated. Under controlled conditions, the use of these biostimulants resulted in a greater soil microbial activity in a 24 h interval with increased soil enzymatic activity. In plants, a positive correlation was found between fertilizers with biostimulant activity and Dualex indices of leaves and content of macronutrients Ca and Mg. In commercial greenhouses, the fertilizers with biostimulant activity strongly depended on the soil texture. In conclusion, these products have real potential to replace conventional fertilizers in commercial production fields.

Keywords: biostimulant; pepper; fertilization; humic substances; root morphology; organic agriculture

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1. Introduction

Conventional farming often results in the reduced biological fertility of soils, decreasing their capacity to support healthy crop growth [1]. It also causes serious environmental problems such as waterway pollution, mineral depletion, soil acidification and agrobiodiversity reduction [2]. Furthermore, organic horticulture has often been reported to be an environmentally friendly production system able to produce food with minimal harm to ecosystems, but with the drawback of a lower yield [3]. The use of natural preparations in agriculture that are not harmful to the environment is particularly important in connection with the progressive processes of soil degradation and atmospheric pollution, which are closely related with global warming [4]. The soil amendment strategy combined with fertilization management generally mitigates CO_2 emissions as a result of retardation of C turnover [5]. The use of organic fertilizers and biostimulants to increase productivity has been shown to be an alternative to reduce the agricultural pressure on the environment [6]. Its use can diminish the effects of environmental abiotic stress factors such as water stress, improve soil water-holding capacity and root conformation, and increase root growth with beneficial effects on nutrient and water use efficiency and yield [7]. They increase plant

development and root growth by, for example, triggering expression of the H^+ATPase, in a similar way to the auxin hormone [8]. When applied to the soil, biostimulants may stimulate rhizosphere microbes and soil enzymatic activity, the photosynthetic process, and the production of hormones or growth regulators in plants [9]. They are beneficial for soil fertility by adsorbing organic solutes, acting as soil pH buffers and complexing metallic ions, thus enhancing micronutrients availability [10]. The humic substances, as biostimulants, improve the soil structure by forming clay–humic complexes, which reduce water infiltration into the aggregates, improving aggregate stability [11].

Other proposed mechanisms have included "indirect action" on the metabolism of the microbial population and the physical conditions of the soil, meaning a better nutrient uptake [12,13]. Microorganisms play a critical role in organic matter degradation, nutrient turnover and pathogen suppression in soils, and are therefore an essential component of sustainable agricultural systems [14]. The microorganisms synthesize a variety of compounds, including polysaccharides, proteins, nucleic acids, carotenoids, lipids, etc., as the products of the decomposition of organic matter [15]. The role of microorganisms in soils results in crop improvements [16].

Plant biostimulant demand has grown exponentially, as has the market around those products, causing Europe and the US to provide frameworks for their regulation [17]. However, the market for biostimulants lacks credibility and is not well established, due to reasons such as lack of research, lack of standard operating procedures to produce biostimulants, long duration (3–5 years) for product development, few patents, low reproducibility of lab results in the field, and bottlenecks in international trading due to strict and highly complicated regulations that vary between countries [18].

The main objective of this work is to evaluate the effectiveness of Micro Carbon Technology (MCT®) fertilizers with biostimulant activity based on digested leonardite (MCT®) in comparison with conventional mineral fertilizers in different soils under controlled conditions and in commercial greenhouses. The agronomical comparison between MCT® and conventional fertilizers was studied through the analysis of enzymatic activities of the soil microorganisms and by the evaluation of different plant parameters, like plant growth, foliar mineral analysis, and chlorophyll indices, of pepper plants (*Capsicum annuum* L.) to test the effectiveness of those products as a replacement for conventional fertilizers.

2. Materials and Methods

2.1. Soil Sampling and Characterization

Soils were obtained from three commercial pepper greenhouses in the Autonomous Community of Murcia (Spain). This Spanish region is one of the most important intensive agricultural regions of Europe, with a total extension of cultivated soil of 411,732 ha. With respect to the pepper crop, the Autonomous Community of Murcia is the second largest producer of pepper in Spain, with an extension of 1531 ha and a pepper production of more than 150,000 t/year, with an approximate value of EUR 129 million. Three soils from three different greenhouses were selected according to different textures: sandy loam (S), clay loam (L) and clay (C). Soil sampling was performed by taking 20 subsamples of each from the 0–20 cm surface layer with an Edelman probe (Eijkelkamp, The Netherlands). The soil samples were air dried for 1 week and sieved to 2 mm before use.

The analysis of the physical-chemical properties of the soils was carried out following official protocols [19]. Briefly, the soil texture was determined by the Boyoucos method, and the pH in a soil suspension ratio of 1:2.5 (w:v) of soil:solution with a Crison GLP 21-pH meter (Hach Lange, Barcelona, Spain). The conductivity in ratio 1:5 (w:v) of soil:solution was determined with a Crison microCM 2200 conductivity meter (Hach Lange, Barcelona, Spain). The soil organic matter content was determined by the potassium dichromate oxidation method. Nitrogen was quantified by the Kjeldahl method and the subsequent analysis of the resulting ammonium by means of colorimetry (Berthelot's method) using the Genesys 10 uV Scanning spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The carbonate content and active limestone in the soil were determined with Bernard's

calcimeter and by extraction with ammonium oxalate and further titration with $KMnO_4$, respectively. The determination of available phosphorus was carried out following the Olsen method, with an extractant solution of 0.5 M $NaHCO_3$, followed by spectrophotometrically quantitation by the Duval method at 660 nm (Thermo Scientific Genesys 10 uV Scanning). The determination of the bioavailable fraction of cations was carried out with NH_4Cl in a ratio of 1:10 (w/v). The final extract was acidified with HNO_3. Ca, Mg and Fe were quantified in the acidified extracts by flame atomic absorption spectrometry, and K was determined by atomic emission (AAnalyst 800, Perkin-Elmer) at wavelengths of 422.7, 285.2, 248.3 and 766.5 nm, respectively.

The pH was slightly basic for all the soils due to the high presence of total limestone (34–43%) (Table 1). The electric conductivity levels of the three soils showed that the L soil presented a higher value which could compromise the crop production. The percentage of organic matter was higher in C soil (3.24%) than in S and L soils (1.90% and 1.58%, respectively). This was also correlated with the C/N ratio, with the C soil having the highest. The soil nutrient assessment showed that Ca values were in the optimal range, but the Mg values were below it (Table 1) [20]. In the opposite case, Fe, K and especially P were found at higher levels. Although multiple factors like chemical, biological, and physical properties and processes influence soil fertility, these soils generally had correct agronomic characteristics [21].

Table 1. Physico-chemical parameters of the studied soils: sandy loam (S), clay loam (L), and clay (C).

	S	**L**	**C**
Sand (%)	71	29	32
Silt (%)	12	29	22
Clay (%)	17	42	46
pH H_2O	8.01	8.27	8.36
pH KCl	7.95	7.85	7.88
Electrical conductivity (dS/m)	0.347	0.952	0.545
Organic matter (%)	1.9	1.58	3.24
Nitrogen (%)	0.09	0.07	0.13
C/N	12.2	13.1	14.5
Total limestone (%)	34	43	43
Active limestone (‰)	45	64	82
Phosphorous (mg/kg)	270	75	121
Potassium (mg/kg)	2.24	4.11	2.74
Calcium (mg/kg)	2.30	1.99	2.05
Magnesium (mg/kg)	0.23	0.28	0.34
Iron (mg/kg)	3.25	5.06	2.31

2.2. Fertilizers and Nutritive Solutions

The Micro Carbon Technology (MCT®) fertilizer products with biostimulant activity [22] are derived from the biological digestion of leonardite. These fertilizers were provided by the company Bio Huma Netics, Inc. (Gilbert, AZ, USA). The leonardite was obtained from a mine in the Northwestern United States and was refined by the company into extremely small carbon- and oxygen-rich organic compounds. All the target products evaluated were liquid formulations based on MCT® combined with inorganic fertilizers (Table 2).

Table 2. Basic properties and nutritional composition of MTC® products.

Product	Nutrient Composition (N-P_2O_5-K_2O) (%)	Density (kg/L)	Total Carbon (%)	pH
TX	6-3-0	1.10	22.4	8.5
TN	12-0-0	1.41	6.7	1.0
TP	0-50-0	1.52	0.05	1.5
TK	0-0-40	1.48	6.5	14
TC	8-0-0 and 15% CaO	1.43	0.4	1.0
TM	8% MgO and 14% S	1.26	1.0	5.0
TS	10% S; 0.5% B; 0.05% Co; 1% Cu; 2% Fe; 1% Mn; 0.05% Mo and 4% Zn	1.31	21.8	2.0

The TX was the product with the higher concentration of MCT® fractions in its composition, and therefore, it was the basic product used to formulate the others.

For the design of conventional fertilizer solutions based only on inorganic salts, the following Panreac (Barcelona, Spain) analytical-grade products were used: $Ca(NO_3)_2{\cdot}4H_2O$, KH_2PO_4, K_2SO_4 and $MgSO_4{\cdot}7H_2O$ as macronutrients and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}H_2O$, H_3BO_3 and $MnSO_4{\cdot}H_2O$ as micronutrients. The KNO_3 used was from Merck & Co., Inc. (Kenilworth, NJ, USA) and Fe-EDDHA from Quimioprox (Barcelona, Spain).

Three different nutrient solutions were designed using the conventional fertilizers and the MCT® products seen above:

- Conventional fertilization (CF): conventional fertilization using only inorganic fertilizers.
- Humic fertilization I (HF): nutrient solution made with the MCT® fertilizers: TP, TM, TC, TN, TK, TS.
- Humic fertilization II (HFX): nutrient solution made with the same MCT® fertilizers of HF plus the TX fertilizer (2 mL/L).

The nutrient concentrations of the all solutions were the following for macronutrients (mmolc/L): N 7.5, P 1, K 4.5, Ca 5, Mg 1.5 and the following for micronutrients (mg/L): Fe 1; Mn 0.5; Cu 0.05; Zn 2; B 0.25; Mo 0.025 [23]. The differences between the treatments were the additional microcarbon with biostimulant effect added with the MTC® biostimulant fertilizers.

2.3. Solutions Effects on the Soil

Three pots were prepared and filled with 1 kg of each soil: S, L and C. After irrigation with deionized water to achieve field capacity, 100 mL of each fertilizer solution was added (CF, HF and HFX). Soil respiration was measured by means of the EGM-4 Environmental Gas Monitor for CO_2 (PP Systems, Amesbury, MA, USA) and the soils were sampled for carrying out the enzymatic tests, at 30 min and at 24 h after the addition of the fertilization treatments. During this time, the pots were incubated in a growth chamber with a constant temperature of 28 °C in darkness.

Soil dehydrogenase activity determination was performed by reducing the triphenyltetrazolium chloride to triphenylformazan [24]. The soil samples (1 g) were mixed with 0.1 M Tris-HCl buffer at pH 7.6 with 1.5% triphenyltetrazolium chloride and the mixture was incubated 24 h at 30 °C. Then, acetone was added, and after shaking and centrifugation, the supernatant absorbance was directly read at 546 nm in the spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) (εTFF = 15.4 mM^{-1} cm^{-1}).

To determine the soil hydrolase, the fluorescein produced from the hydrolysis of fluorescein diacetate was measured [25]. The soil sample (1 g) was taken and mixed with 60 mM KH_2PO_4/K_2HPO_4 buffer at pH 7.6. The fluorescein diacetate solution was added and incubated 20 min at 30 °C. After that time, acetone was added to stop the reaction and then centrifuged after a manual stirring. Supernatant absorbance was directly measured at 490 nm in the spectrophotometer (εTFF = 80.3 M^{-1} cm^{-1}).

The soil enzymatic activity urease was determined by the formation of NH_4^+ from urea [26]. The soil sample (1 g) was incubated 2 h at 37 °C with 0.1 M urea and 0.1 M borate buffer pH 8.8. Then, 1.35 M KCl in 0.1 M HCl solution was added, and the suspension was stirred. The samples were centrifuged and the NH_4^+ was determined colorimetrically in the supernatant [27].

2.4. Solutions Effects on Pepper Plant

2.4.1. Assay in Controlled Conditions

The agronomic assay in growth chamber was carried out under controlled parameters to test the efficiency of the MCT® fertilizers compared to conventional inorganic fertilizers on pepper plants (*Capsicum annuum* L. cv. Brocanto). Pepper plants were grown from 45-day-old seedlings provided by a commercial seedling supplier (Surinver Coop. V., Murcia, Spain). The seedlings were individually placed in pots with 500 g of each soil (S, L and C) in the growth chamber. A maximum temperature of 28 °C and a minimum of 19 °C were programmed, and relative humidity was controlled to 40% and 60%, with a day/night cycle of 14 and 10 h, respectively. The three nutrient solution treatments (CF, HF and HFX) were supplied by self-compensating drippers of 2 L/h with an average volume of 100 mL per plant and day. The assay was performed in triplicate with one plant per pot. After 62 days of cultivation, the plants were sampled and analyzed.

To quantify plant development, the plant weights, percentage of leaf organic matter and foliar radiometric indices of anthocyanins (Anth), flavonols (Flav), chlorophylls (Chl) and nitrogen balance index (NBI) (ratio: chorophyll/flavonols activities) obtained by means of the DUALEX Scientific + TM meter (ForceA, Paris, France) were used. The nutritional status of the plant was measured by foliar analysis. Leaves were dried in a forced air oven at 65 °C for 3 days, and weighted. The foliar mineral content was analyzed after dry digestion at 480 °C for 2 h and further acid digestion for ash solubilization with HCl 6 M at 90 °C [28]. The elements were quantified by ICP-MS (NexION 300XX, Perkin-Elmer, Waltham, MA, USA). Foliar nitrogen was quantified by colorimetry (Berthelot's method) with spectrophotometry at 660 nm (ThermoFisher Scientific, Waltham, MA, USA) after Kjeldahl digestion of leaves.

2.4.2. Field Conditions in Commercial Greenhouses

The agronomic assay was carried out in three commercial greenhouses in the Community of Murcia (Spain). The greenhouses had a surface area of 0.6 ha with a plant density of 25 K plants/ha with 25 cm between plants and 1 m between lines. The pepper crop was planted the first week of December 2018 with the first addition of the nutritive products the last week of January 2019. The irrigation was conducted by drips during 1 h with a volume of 50 K L/ha with a fertilizer solution dose of 0.4 mL/L. The solution used contained, in mmolc/L: 5 NO_3^-, 1.3 NH_4^+, 2 P_2O_5, 0.2 K^+ and 1 Ca^{2+}. The sampling was performed at the end of June 2019, coinciding with commercial harvesting. Vegetable sampling was carried out, taking 4 plants randomly from each greenhouse at the end of the production campaign (5 months in total). Whole plants were taken, including the root that was extracted by undermining the root zone with a hoe. The greenhouses chosen were the sampling points of the soil used for the previous assay (S and C). These plants were called PS and PC; and corresponded to fully grown plants harvested for commercial purposes and fertilized entirely with the MCT® products. Plants of the greenhouse L were not sampled, because this greenhouse faced several problems related with pepper production.

Additionally, plants from two other greenhouses with the same fertilizer regime but with a different type of soil from the previous ones were sampled and were called PCF (plants with a conventional fertilizing treatment) and PHF (plants with an MCT® fertilizing treatment during their growing cycle in the greenhouse). The quantification of plant growth was carried out in the same way as described in Section 2.4.1.

2.5. Statistical Analysis

The data were statistically evaluated by means of the analysis of the variance with ANOVA two-way followed by Duncan's post hoc test with a level of significance of 95% ($p \leq 0.05$) to find significant differences between treatments, using the software IBM SPSS Statistical Package for the Social Sciences v20. The principal component analysis was carried out by the PAST V. 4.02 software (Natural History Museum, University of Oslo).

3. Results

3.1. Effects of Nutrient Solutions on the Soil

All the treatments resulted in a decrease in soil respiration at 24 h irrespective of the tested soils (Figure 1). However, more noticeable differences were found for the soil enzymatic activity increments among treatments. Increment of dehydrogenase activity for all the fertilization treatments were found for the clay (C) soil. For the rest of the soils, the HF treatment (MCT® products) was the only one which produced an important increase in the dehydrogenase activity. On the contrary, soil hydrolase activity showed increments at 24 h for all the treatments and soils except for LHFX. Interestingly, the tendency was the opposite to that of dehydrogenase, with the MCT® fertilizers inducing higher enzymatic activities in the S and L soils but less on the C soil (Figure 1). Soil urease activity increased after the addition of the fertilization treatments in the S soil, but not in the rest of the soils (except for the CHF treatment). In S soil, the addition of the HFX solution resulted in the highest increment.

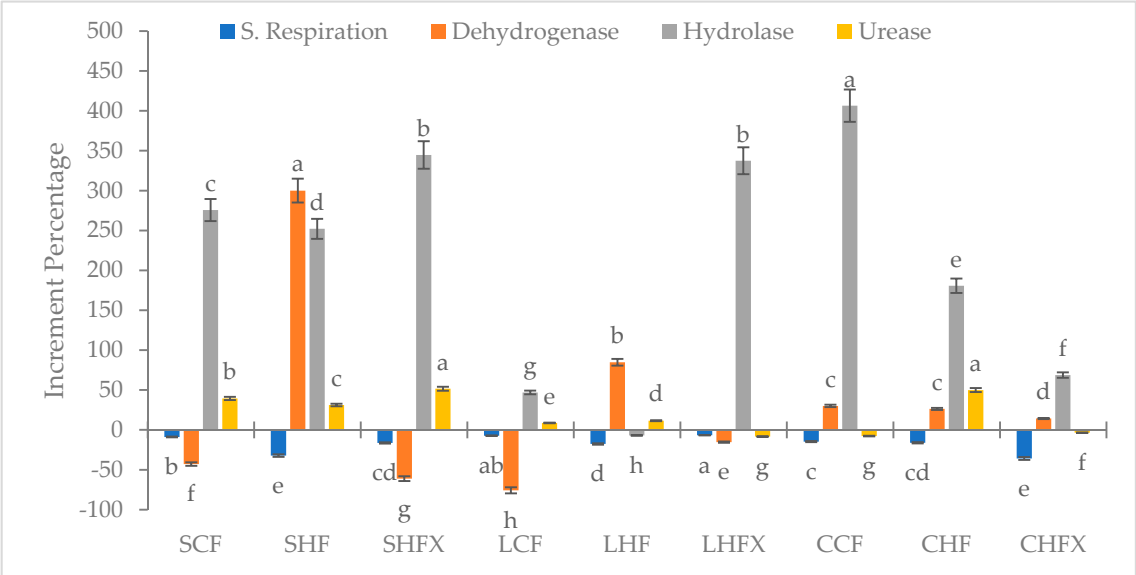

Figure 1. Increment (30 min to 24 h) of soil respiration, dehydrogenase activity, hydrolase activity and urease activity in the soils: sandy loam (S), clay loam (L) and clay (C) soils using the nutrient solutions: conventional fertilization (CF), MCT® fertilizers (HF) and MCT® fertilizers plus TX (HFX). Different letters indicate significant differences between treatments (n = 3; Duncan's test, $p < 0.05$).

3.2. Nutrient Solution Effects on Pepper

3.2.1. Controlled Conditions in Growth Chamber

The type of soil produced significant effects on the final weight of leaves and stem. However, the fertilization treatments were not a significant factor for the plant growth (Table 3). There was not a significant interaction between fertilization treatments and soil type. The plants grown in the L soil presented the lowest leaf and stem weights. However, root and fruit production did not show significant differences among the fertilization treatments or soils. For the CHFX treatment, fruit production was not measured because of an important delay in the fructification.

Table 3. Fresh weight of leaves, stem, root, and fruit of *Capsicum annum* L. grown in the sandy loam (S), clay loam (L) and clay (C) soils using the nutrient solutions: conventional fertilization (CF), MCT® fertilizers (HF) and MCT® fertilizers plus TX (HFX). Data are presented in grams as the mean ± standard deviation (n = 3). Two-way ANOVA was performed to evaluate the effect of fertilization (F) and soil (S) and their interaction.

	SCF	SHF	SHFX	LCF	LHF	LHFX	CCF	CHF	CHFX	F	S	FxS
						g				Two-Way ANOVA		
Leaves	29 ± 13	35 ± 7	17 ± 4	6 ± 8	24 ± 16	11.7 ± 0.8	26.3 ± 0.3	32 ± 4	37 ± 13	N.S.	**	N.S.
Stem	21 ± 13	25 ± 4	16 ± 5	8 ± 11	17 ± 10	9 ± 1	18 ± 5	19 ± 4	30 ± 7	N.S.	*	N.S.
Root	139 ± 101	136 ± 9	137 ± 51	63 ± 90	190 ± 102	82 ± 48	142 ± 112	171 ± 117	267 ± 76	N.S.	N.S.	N.S.
Fruit	49 ± 69	6.5 ± 0.4	68 ± 59	1 ± 1	27 ± 24	33 ± 2	30 ± 37	73 ± 38	-	N.S.	N.S.	N.S.

N.S. not significant; * $p < 0.05$; ** $p < 0.01$. F: fertilizer, S: soil and F × S: its interaction.

Spectrometric leaf indices given by Dualex showed significant differences for all the soils, the fertilization treatments, and their interaction (Table 4). The plants grown in C soil showed the highest values of NBI, with no differences between the HFX and CF treatments. For the rest of the soils, the treatment HFX resulted in the lowest NBI values. For the L soil, the HF treatment gave higher NBI values than CF. The chlorophyll activities followed the same pattern with the highest values for the C soil and the lowest value for the LHFX treatment. However, this treatment produced the highest value of flavonols and anthocyanins indices (Flav and Anth), followed by the LCF treatment. No differences were found between the treatments CF, HF and HFX in the C and S soils.

Table 4. Dualex indices of Nitrogen Balance Index (NBI), chlorophylls (Chl), flavonols (Flav) and anthocyanins (Anth) of *Capsicum annum* L. grown in the sandy loam (S), clay loam (L) and clay (C) soils using the nutrient solutions: conventional fertilization (CF), MCT® fertilizers (HF) and MCT® fertilizers plus TX (HFX). Data are presented as the mean ± standard deviation (n = 3). Two-way ANOVA was performed to evaluate the effect of fertilization (F) and soil (S) and their interaction.

	SCF	SHF	SHFX	LCF	LHF	LHFX	CCF	CHF	CHFX	F	S	FxS
										Two-Way Anova		
NBI	60 ± 9^{cd}	68 ± 12^{bc}	51 ± 19^{de}	45 ± 8^{e}	58 ± 10^{cd}	23 ± 9^{f}	81 ± 13^{a}	68 ± 13^{bc}	73 ± 11^{ab}	***	***	***
Chl	41 ± 3^{bcd}	40 ± 3^{cde}	37 ± 9^{de}	36 ± 5^{e}	37 ± 6^{de}	22 ± 7^{f}	48 ± 4^{a}	45 ± 3^{abc}	46 ± 5^{ab}	***	***	***
Flav	0.69 ± 0.08^{cd}	0.61 ± 0.08^{d}	0.8 ± 0.1^{bc}	$0.81 \pm 0.08_{b}$	$0.64 \pm 0.08_{d}$	1.0 ± 0.1^{a}	0.6 ± 0.08^{d}	0.7 ± 0.1^{cd}	$0.63 \pm 0.05_{d}$	***	***	***
Anth	$0.053 \pm 0.005_{bcd}$	$0.051 \pm 0.003_{bcd}$	$0.06 \pm 0.02_{bc}$	$0.06 \pm 0.01_{b}$	$0.06 \pm 0.01_{bc}$	$0.11 \pm 0.02_{a}$	$0.047 \pm 0.009_{cd}$	$0.042 \pm 0.006_{d}$	0.043 ± 0.008^{d}	***	***	***

Different letters indicate significant differences between treatments (n = 3; Duncan's test, $p < 0.05$). N.S. not significant; *** $p < 0.001$.

Foliar analysis showed significant differences between treatments and soils (Table 5). The interaction fertilization × soil was significant for Ca and Mg. For both nutrients, the treatment which showed the least content in leaves was the conventional inorganic solution in the clay loam soil (LCF). In this soil, with the worst agronomic characteristics (Table 1), the use of the biostimulant solutions HF and HFX increased the content of Ca and Mg in plant leaves with respect to conventional fertilization. For the other macronutrients (N, P and K) there were no significant differences between soils or fertilization treatments. Hence, the nutritional ability of HF and HFX treatments was comparable to conventional inorganic fertilization. However, all the micronutrients except Fe and Mn were significantly affected by the fertilization treatments. The treatments HF and HFX, in general, increased the leaves content of the micronutrients B, Co, Cu, Mo and Zn. For all fertilization treatments, no significant differences in nutrient content were observed between soils, except for B, Fe and Mo. B was higher for the plants grown in the L soil and Mo in the C soil. The foliar concentration of Fe was significantly affected by the soil used but not by the fertilization treatment, with the highest values measured for the C soil. No interaction fertilization × soil was found for the micronutrients.

Table 5. Foliar macro and micronutrient analysis of *Capsicum annum* L. grown in the sandy loam (S), clay loam (L) and clay (C) soils using the nutrient solutions: conventional fertilization (CF), MCT® fertilizers (HF) and MCT® fertilizers plus TX (HFX). Data are presented as the mean ± standard deviation (n = 3). Two-way ANOVA was performed to evaluate the effect of fertilization (F) and soil (S) and their interaction.

	SCF	SHF	SHFX	LCF	LHF	LHFX	CCF	CHF	CHFX	F	S	FxS
					$g \cdot kg^{-1}$					Two Way ANOVA		
N	57 ± 25^{a}	41 ± 4^{ab}	46 ± 12^{ab}	24 ± 33^{b}	42 ± 14^{ab}	43 ± 2^{ab}	49 ± 4^{ab}	54 ± 9^{ab}	53 ± 13^{ab}	N.S.	N.S.	N.S.
P	0.67 ± 0.05^{ab}	0.5 ± 0.1^{ab}	0.8 ± 0.3^{ab}	0.3 ± 0.5^{b}	0.58 ± 0.08^{ab}	0.44 ± 0.05^{ab}	0.6 ± 0.2^{ab}	0.8 ± 0.3^{a}	0.9 ± 0.1^{a}	N.S.	N.S.	N.S.
K	46 ± 2^{a}	41 ± 4^{a}	42 ± 5^{a}	26 ± 36^{a}	39 ± 12^{a}	43 ± 9^{a}	43 ± 11^{a}	43 ± 5^{a}	43 ± 2^{a}	N.S.	N.S.	N.S.
Ca	34 ± 7^{a}	42 ± 3^{a}	36 ± 9^{a}	10 ± 15^{b}	40 ± 9^{a}	33 ± 2^{a}	35 ± 12^{a}	29 ± 3^{a}	30 ± 4^{a}	N.S.	N.S.	*
Mg	11 ± 1^{a}	15 ± 2^{a}	12 ± 3^{a}	4 ± 6^{b}	15 ± 2^{a}	13 ± 1^{a}	11 ± 1^{a}	10.2 ± 0.6^{a}	10.4 ± 0.6^{a}	*	N.S.	*
					$mg \cdot kg^{-1}$							
B	$128 \pm 68_{bcd}$	$151 \pm 15_{bcd}$	199 ± 68^{ab}	54 ± 76^{d}	169 ± 76^{bc}	278 ± 65^{a}	74 ± 29^{cd}	94 ± 17^{bcd}	102 ± 9^{bcd}	**	*	N.S.
Co	$0.7 \pm 0.4_{cd}$	$1.0 \pm 0.3_{cd}$	2.3 ± 0.9^{ab}	0.3 ± 0.4^{d}	2 ± 1^{ab}	2.8 ± 0.1^{a}	$0.84 \pm 0.05_{cd}$	$1.4 \pm 0.3_{bcd}$	$1.7 \pm 0.3_{abc}$	***	N.S.	N.S.
Cu	19 ± 4^{ab}	18 ± 3^{ab}	26 ± 13^{a}	5 ± 8^{b}	15 ± 7^{ab}	26 ± 5^{a}	17 ± 6^{ab}	13 ± 3^{ab}	17 ± 3^{ab}	*	N.S.	N.S.
Fe	$61 \pm 19_{abc}$	46 ± 3^{bc}	54 ± 10^{abc}	30 ± 43^{c}	45 ± 4^{bc}	$41.3 \pm 0.2_{bc}$	85 ± 26^{a}	67 ± 9^{abc}	72 ± 14^{ab}	N.S.	**	N.S.
Mn	$113 \pm 56_{abc}$	$95 \pm 26_{bc}$	213 ± 95^{a}	21 ± 30^{c}	$160 \pm 103_{ab}$	112 ± 9^{abc}	85 ± 14^{bc}	90 ± 4^{bc}	$111 \pm 29_{abc}$	N.S.	N.S.	N.S.
Mo	$1.9 \pm 0.7_{abc}$	$2.0 \pm 0.2_{ab}$	$1.33 \pm 0.05_{bc}$	0.5 ± 0.7^{d}	$1.9 \pm 0.5_{abc}$	1.1 ± 0.3^{cd}	2.1 ± 0.2^{ab}	2.7 ± 0.3^{a}	1.6 ± 0.3^{bc}	**	**	N.S.
Zn	$127 \pm 12_{bcd}$	$135 \pm 35_{abcd}$	200 ± 75^{ab}	36 ± 50^{d}	$105 \pm 51_{bcd}$	232 ± 76^{a}	$106 \pm 25_{bcd}$	95 ± 25^{cd}	$140 \pm 25_{abc}$	**	N.S.	N.S.

Different letters indicate significant differences between treatments (n = 3; Duncan's test, $p < 0.05$). N.S. not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2.2. Field Conditions in Commercial Greenhouses

The analysis of pepper plants in commercial greenhouses showed differences between soils but not between fertilization treatments (Table 6). The C soil produced lower fruit hardness. The plants grown in this soil also had lower levels of the Dualex indices of NBI and Chl. For all the soils, at real scale, fertilization with the MCT® biostimulant products resulted in the same production and plant health status as conventional inorganic fertilization. The only parameters that depended on the fertilization were the content of Flav and O.M., which were both lower when the conventional inorganic solution was used.

Table 6. Plant growth values of parts weights, fruit hardness, Dualex parameters of Nitrogen Balance Index (NBI), clorophylls (Chl), flavonols (Flav) and anthocyanins (Anth) and organic matter percentage of pepper plants grown in commercial greenhouses in the C soil (PC) and S soil (PS) and with different solutions PCF (conventional treatment) and PHF (plants with an MCT® treatment). Data are presented as the mean ± standard deviation. Different letters indicate significant differences between treatments (n = 4; Duncan's test, $p < 0.05$).

	PCF	PHF	PS	PC
		Morphological parameters		
Leaves (g)	420 ± 20^{a}	444 ± 24^{a}	447 ± 86^{a}	347 ± 170^{a}
Stem (g)	388 ± 38^{a}	410 ± 30^{a}	448 ± 49^{a}	422 ± 233^{a}
Fruit (g)	702 ± 616^{a}	1580 ± 278^{a}	1075 ± 774^{a}	747 ± 359^{a}
Fruit hardness	3 ± 1^{a}	2.9 ± 0.8^{a}	2.9 ± 0.9^{a}	2.0 ± 0.9^{b}
		Chemical parameters		
NBI	52 ± 17^{a}	45 ± 16^{a}	36 ± 11^{b}	29 ± 7^{c}
Chl	41 ± 8^{a}	43 ± 6^{a}	41 ± 7^{a}	36 ± 6^{b}
Flav	0.8 ± 0.2^{c}	1 ± 0.3^{b}	1.2 ± 0.4^{a}	1.3 ± 0.3^{a}
Anth	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}
O.M. (%)	73.8 ± 0.8^{b}	74.7 ± 0.8^{ab}	75.2 ± 0.6^{ab}	76 ± 2^{a}

Differences in leaf nutrient content depended more on the type of soil used than on the fertilization followed (Table 7). The biostimulant instead caused lower content of Mg in leaves compared with conventional fertilization only in one soil, but not for the S and C soils. The clay soil presented the highest nutrient concentration differences, with lower levels of the macronutrients P and K but higher levels of the micronutrients B, Co and Zn. The sandy loam soil (PS), on the other hand, resulted in lower levels of the micronutrient Mn and higher levels of Mo. For the rest of the nutrients, fertilization with the MTC® products achieved the same results as conventional fertilization with inorganic salts.

Table 7. Nutrient concentration of pepper plants cv. Brocanto grown in commercial greenhouses in the C soil (PC) and S soil (PS) treated with MCT® products and with different solutions PCF (conventional treatment) and PHF (plants with MCT® treatment). Data are presented as the mean ± standard deviation. Different letters indicate significant differences between treatments (n = 4; Duncan's test, $p < 0.05$).

	PCF	PHF	PS	PC
		$g \cdot kg^{-1}$		
N	36 ± 2 ^{a}	38 ± 5 ^{a}	34 ± 4 ^{a}	35 ± 2 ^{a}
P	0.68 ± 0.09 ^{a}	0.7 ± 0.1 ^{a}	0.70 ± 0.06 ^{a}	0.53 ± 0.03 ^{b}
K	41 ± 5 ^{ab}	47 ± 1 ^{a}	43 ± 3 ^{a}	36 ± 5 ^{b}
Ca	47 ± 4 ^{a}	43 ± 3 ^{a}	47 ± 1 ^{a}	41 ± 7 ^{a}
Mg	16 ± 1 ^{a}	13 ± 1 ^{b}	16 ± 1 ^{a}	15 ± 2 ^{ab}
		$mg \cdot kg^{-1}$		
B	147 ± 36 ^{b}	106 ± 40 ^{b}	111 ± 4 ^{b}	201 ± 39 ^{a}
Co	2.1 ± 0.7 ^{b}	2.7 ± 0.6 ^{b}	1.2 ± 0.2 ^{c}	3.8 ± 0.5 ^{a}
Cu	16 ± 7 ^{a}	19 ± 5 ^{a}	21 ± 2 ^{a}	19 ± 4 ^{a}
Fe	137 ± 34 ^{a}	154 ± 49 ^{a}	148 ± 40 ^{a}	146 ± 16 ^{a}
Mn	227 ± 72 ^{a}	268 ± 45 ^{a}	129 ± 18 ^{b}	240 ± 45 ^{a}
Mo	0.7 ± 0.2 ^{b}	0.7 ± 0.1 ^{b}	1.0 ± 0.2 ^{a}	0.59 ± 0.06 ^{b}
Zn	135 ± 32 ^{ab}	125 ± 21 ^{ab}	120 ± 2 ^{b}	139 ± 6 ^{a}

4. Discussion

The S soil, with a sandy-loam texture (Table 1), allowed a better aeration of the soil, in contrast to the C soil with clay texture, small size of particles, high compactness, and low permeability, leading to possible waterlogging after irrigation with less soil aeration. The L soil had a loam texture, which is the intermediate point of the characteristics of the other soils. High pH values may influence the addition of fertilizers, as this feature can alter them, causing precipitation and reducing the availability, for example, of Fe [29] or P [30]. Pepper crops specifically tolerate slightly basic pH, so this factor could not be limiting for subsequent trials. The high C/N ratio of the soils L and C due to the low N content resulted in an excess in the energy of the soils, which could enhance microbial activity and respiration [15]. The high concentration values of some nutrients in the soil could be related to possible overfertilization with continued addition of K and P, resulting in them being retained in the soils.

Dehydrogenases are the enzymes that catalyze the reduction or oxidation of a substrate by hydrogen. It is the most representative soil enzyme among soil oxidoreductases, and its activity is one of the most used parameters for evaluating the microbiological status of the soil [31]. Since increments were found after the addition of biostimulant products (Figure 1), a positive effect was found on the soil microorganisms. Hydrolases are enzymes capable of catalyzing the hydrolysis of a chemical bond using water. In the soil, these enzymes are used for the degradation of complex organic matter, generating molecules that are more easily accessible to soil organisms [32]. The increase in the activity of these enzymes, in the S and L soils (Figure 1), meant that the soil microorganisms were activated by MTC® products. This could be led by better nutrition for crops through the generation of potentially useful

degradation compounds [33] from MCT® or soil organic matter. Urease is the enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia. A large increase in the activity of this enzyme in a short period of time results in the loss of urea fertilizers due to its volatilization as ammonia, causing environmental pollution problems [34]. Since the highest increment was found for the SHFX treatment (Figure 1), the organic matter included in the TX product affected the microbiota in soils with good aeration.

Remarkably, the greatest increases in activities occurred in the S soil. This soil had a sandy loam texture compared to the rest, whose texture was more clayey (Table 1). This texture seems to be the most suitable for the growth of microorganisms [35] due to the soil being less compact, and therefore having better aeration. Another influential factor is the C/N ratio of the soil [36]. The S soil presents the most suitable value for the C/N ratio. The other soils possessed higher C/N ratios, with a smaller amount of N in proportion, negatively affecting the activity of the microorganisms. In general, soils irrigated with HFX had a more greatly increased percentage of hydrolase activity than soils irrigated with HF. This can be explained by the fact that the HFX solution contained the product TX, which included free amino acids [22], and these are the preferred form of nitrogen by the soil microbiota [37]. However, the opposite trend was observed in the C soil, with a higher increase in enzymatic activities when using HF instead of HFX. This fact could be associated with its clay texture, which could prevent the correct development of microorganisms in the soil. Therefore, the amount of carbon and nitrogen added in the form of MCT® could not have been used. The L soil showed the lowest increments in enzymatic activities probably because of the relative unsuitable agronomic parameters due to its higher E.C. (Table 1). The higher conductivity of L soil negatively affected the soil microbiota, leading to the worst conditions for the rhizosphere ecosystem.

Soil enzymes take part in the circulation of biogenic elements C, N, P, and S, which is of great importance in terms of ecology, as the activity of microorganisms and the enzymes they secrete provides the cycle of elements to nature [38]. The MCT® generated, in general, a greater microbial activity in a 24 h interval, resulting in an increase in enzymatic activity. However, soil properties are a key factor, since the texture and the C/N ratio and E.C. are still essential [39].

The results of the assay in controlled conditions showed the lowest plant growth for the L soil (Table 3). This lower growth could be due to the greater E.C. that this soil presented in comparison to the other soils (Table 1). High conductivities cause serious problems for the growth, development and survival of plants by inducing osmotic and specific ion toxicity, causing a reduction in growth, imbalances or nutritional deficiencies, and symptoms of toxicity, reducing the yield of the fruit and reducing the quality, with the growth and the production being closely related to the water and the ionic state of the plant in particular [40]. For the other soils, fertilization treatments did not result in significant differences in the plant growth, meaning that the treatments HF and HFX had the same efficiency as the conventional inorganic fertilization (CF). Fruit production was, although not significantly, higher when the MTC® products were used.

The parameter of chlorophylls (Chl) can be related to the nutritional status of the plant with respect to nitrogen [41], being high for both the conventional and the biostimulant treatments. These products also resulted in an improvement of the plant health status in terms of foliar activities, with the highest contents of flavonols and anthocyanins being found for the HFX treatment (Table 4). In plants treated with biostimulants, antioxidant contents are higher compared to non-treated plants [42]. Biostimulants are able to increase the color of leaves by stimulating chlorophyll biosynthesis and inducing phenolic and flavonoid metabolism in horticultural crops [43]. Leaf color is an important quality parameter in vegetable crops and is related to the chlorophyll activity of leaves [44]. The chlorophyll content is also related to stress physiology and abiotic factors, like light and water status, which are essential in primary production [45].

Since HF and HFX treatments increased the nutritional content of plant leaves grown in L soil, a positive effect was found in soils with worse agronomic characteristics. Bios-

timulants can increase the concentration of several macronutrients in the shoots during generative development [46]. In summary, the soil factor is a key factor, and greatly interferes with the effectiveness of fertilizers, as has been reported previously [47]. If the agronomic characteristics of the soil are adequate, conventional fertilization generates productions comparable to those obtained with the new fertilizers tested (MCT®). In the L soil with a higher conductivity value, the fertilization with MCT® resulted in equal pepper production to the conventional inorganic fertilization but a better plant status.

In commercial greenhouses, although the leaf nutrient content was adequate [48], differences were less clear between the fertilization treatments. These data were in agreement with those obtained by Russo et al. [49], which showed that treatment of a crop with humic acids did not have a significant effect on the firmness of the fruit, the length of the fruit, or the diameter, but it did significantly influence the total content of chlorophyll b and resulted in a significantly higher average fruit weight. However, the high variability of production in the PHF treatment resulted in a loss of significant differences between conventional fertilization and MCT® fertilization (Table 6). In agronomy, one of the most important parameters is the yield, along with the equilibrium between amount and quality. The high nutrient concentration in leaves is less important if the fruit production or the quality are not affected. In our study, the MCT® fertilizers resulted in similar nutritional contents and fruit production to conventional inorganic fertilization in pepper plants in commercial greenhouses. This means that MTC® products are at least comparable to conventional fertilization in agronomic conditions.

5. Conclusions

Fertilization based on MCT® products resulted in significant improvements in the pepper crop production, with the effects being more noticeable in unfavorable conditions of higher soil conductivity. Under favorable agronomic conditions, these products had comparable effects to those of conventional inorganic fertilization. The efficiency of these products is strongly affected and defined by the soil texture. Although more trials are needed to clarify the mechanisms of action of these biostimulants on plants, the results on plant growth and development in a controlled and in commercial greenhouses showed that the use of MCT® products, improved plant adaptation under abiotic stress conditions leading to comparable fruit production than a conventional fertilization. However, more research is needed in terms of economic viability of its use in commercial greenhouses production.

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