

## Article

# Influence of Formulation on the Cuticular Penetration and on Spray Deposit Properties of Manganese and Zinc Foliar Fertilizers

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**Abstract:** Foliar fertilization, or the application of nutrient solutions to the foliage of plants, has become a very important tool as a supplement to traditional soil fertilization. So far, knowledge about the real mechanisms of foliar nutrient uptake is still limited. In this study different manganese (Mn) and zinc (Zn) carriers differing in their solubility and chemical characteristics (chelated or non-chelated, with or without the presence of a surfactant-penetrant) were compared with regard to their penetration characteristics through enzymatically-isolated cuticles. The experiments were explicitly conducted under high humidity conditions in order not to penalize compounds with a higher deliquescent point. The results show that Mn penetrates more rapidly through the cuticle than Zn ions for unknown reasons. The addition of a surfactant-penetrant enhances the penetration rate in the case of Mn ions. This trend is much less pronounced for zinc ions. Formulations based on insoluble carriers, such as carbonate or oxide, only poorly penetrate through the cuticle. In order to rapidly control micronutrient deficiency problems, only fully water soluble micronutrient carriers should be used.

**Keywords:** foliar fertilization; manganese; zinc; isolated cuticles; surfactant-penetrant; chelates

## 1. Introduction

Foliar fertilization has become, after many decades of research and development, an important tool for the sustainable and productive management of crops and is of significant commercial importance world-wide [1–4]. In addition to obtaining more practical experience about the optimum stages of foliar fertilizer application [2], little is known about the possibilities to improve foliar nutrient penetration into leaf or fruit tissues.

As experimental field trials often provide inconsistent results depending on the specific climatic conditions of single seasons, the study of the foliar penetration efficacy of foliar nutrients has long been extended to model systems based on the use of isolated astomatous cuticles [3,5–7]. Any study of the mechanism and efficacy of foliar absorption must consider ion penetration of the cuticle. The cuticle, or cuticular membrane, is the first and most important barrier to be overcome before a foliar fertilizer can be absorbed into the cytoplasm and integrated into plant metabolism [3,7,8]. The mechanisms of cuticular penetration of polar and hydrophilic compounds, as usually contained in foliar fertilizers, are currently not fully understood [9]. The cuticle is a lipid semipermeable membrane where the existence of aqueous pores [5,6] has been suggested as a pathway of penetration of ionic or hydrophilic compounds, such as nutrient ions. Although these aqueous pores in the cuticle cannot be evidenced by electron microscope or other optical techniques, sophisticated ion permeability studies provide strong evidence of their existence [3]. Any penetration of compounds through these aqueous pores

implicates that such compounds or nutrients must be dissolved in water [3,5,6]. Water solubility is a key factor for foliar uptake, since absorption will occur only when the applied compound is dissolved in a liquid phase—usually water—and will subsequently diffuse into the plant organs [3,6]. In addition to water solubility, another fundamental prerequisite of a nutrient compound is its molecular size. The cuticular membrane and its aqueous pores are size-selective, and only molecules up to a certain size are able to penetrate through cuticular pores [3]. This is true for cuticular pores, as well as for stomatal pores, which are several times larger—an additional pathway for entry of solutes into the leaf [3,10–13]. Cationic micronutrients are commonly used as chelates and the benefit of chelation as to improved absorption and translocation is discussed very controversially [3,8,14]. Less penetration of chelated Fe, Mn, and Zn through isolated tomato cuticles was observed than the salt forms, but further translocation of the chelates in the tissues was much higher [8]. Unfortunately, the relative humidity conditions during the experiments, and its crucial role in cuticular penetration [10,15,16] are rarely defined, i.e., controlled in the experiments.

The objective of the present studies was to evaluate the penetration of different commercial Mn and Zn foliar fertilizers through enzymatically-isolated tomato cuticles. The selected foliar fertilizers contain zinc or manganese with different water solubility (fully-soluble salts and chelates, as well as insoluble oxides or carbonates) and in a chelated or non-chelated state. Studies on the cuticular penetration were accompanied by scanning electron microscopy analysis (SEM) of the structure of the cuticular deposits of the single foliar fertilizers.

## 2. Experimental Section

### 2.1. Plant Material and Isolation of Cuticular Membranes

The studies were conducted under controlled conditions at the University of Bonn, INRES-Horticultural Sciences. Tomato plants (*Lycopersicon esculentum*) of the cultivar Capricia (Rijk Zwaan Welter GmbH, Germany) were grown without any application of pesticides or foliar fertilizers in a commercial-like greenhouse at the experimental station Campus Klein-Altendorf (University of Bonn, Bonn, Germany). Fully-ripe fruits were carefully harvested, transported to the lab, and used for the enzymatic separation of cuticular membranes. Disks (25 mm diameter) were punched out from fruits and leaves with a cork borer. Cuticular membranes were enzymatically isolated using cellulase (20 mL · L<sup>-1</sup> Celluclast, National Centre for Biotechnology Education, The University of Reading, Reading, UK) and pectinase (20 mL · L<sup>-1</sup> Trenolin® Flot DF, Erbsloeh Geisenheim AG, Geisenheim, Germany), 14.7 g · L<sup>-1</sup> tri-Sodium citrate-dihydrate and 0.068 g · L<sup>-1</sup> NaN<sub>3</sub> (Sodium azide) for preventing microbial growth. The pH of the enzymatic solution was regulated to a range between 3.5 and 4. The solution was first changed after seven days; thereafter, a new solution every 10–14 days. After approximately 50 days, when cuticles were completely free from cell walls, cuticular membranes were rinsed with distilled water and transferred into a Borax-buffer solution (pH = 9) for stopping enzyme activities, and stored in this buffer solution for another five days. Thereafter, cuticles were removed from the buffer solution, washed with distilled and deionized water, and dried at room temperature for two days before dry-storing in closed Petri dishes. Before each experiment, cuticles were checked for their integrity using a stereo microscope. Cuticles with changed structure would have ruptures and would be excluded from further evaluations.

### 2.2. Treatment Solutions

A detailed list of the treatments is provided in Table 1. The commercial suspension formulations as well as the Mn- and Zn sulfates were used at equivalent metal concentration of 600 mg · L<sup>-1</sup>, with the exception of Zn-EDTA Plus, which was used differently as originally scheduled (136 mg · L<sup>-1</sup> Zn). The “Plus” formulations contain a novel proprietary penetration enhancer adjuvant (belonging to the chemical class of alkoxyated non-ionic alkanols) in order to increase their effectiveness.

**Table 1.** Overview of the Mn and Zn formulations used in the experiments; deionized water served as the control treatment.

Formulation	Physical Form	Contents % w/w Zn or Mn	Water Solubility and Chelation
H <sub>2</sub> O	Deionized water	-	-
Mn-EDTA	Suspension	6.0% Mn	Soluble; Mn chelated by EDTA
Mn-EDTA Plus	Suspension	6.0% Mn + penetration enhancer	Soluble; Mn chelated by EDTA
Zn-Gluconate	Suspension	6.0% Zn	Soluble; Zn chelated by Gluconic acid
Zn-Gluconate Plus	Suspension	6.0% Zn + penetration enhancer	Soluble; Zn chelated by Gluconic acid
Zn-EDTA	Suspension	8.0% Zn	Soluble; Zn chelated by EDTA
Zn-EDTA Plus	Suspension	8.0% Zn + penetration enhancer	Soluble; Zn chelated by EDTA
Zn Oxide	Suspension	40% Zn as zinc oxide	Water insoluble Zn
Mn Carbonate & Oxide	Suspension	27.4% Mn as manganese carbonate & oxide	Water insoluble Mn
Mn sulfate	Unformulated salt	32% Mn as sulfate	Soluble Mn
Zn sulfate	Unformulated salt	35% Zn as sulfate	Soluble Zn

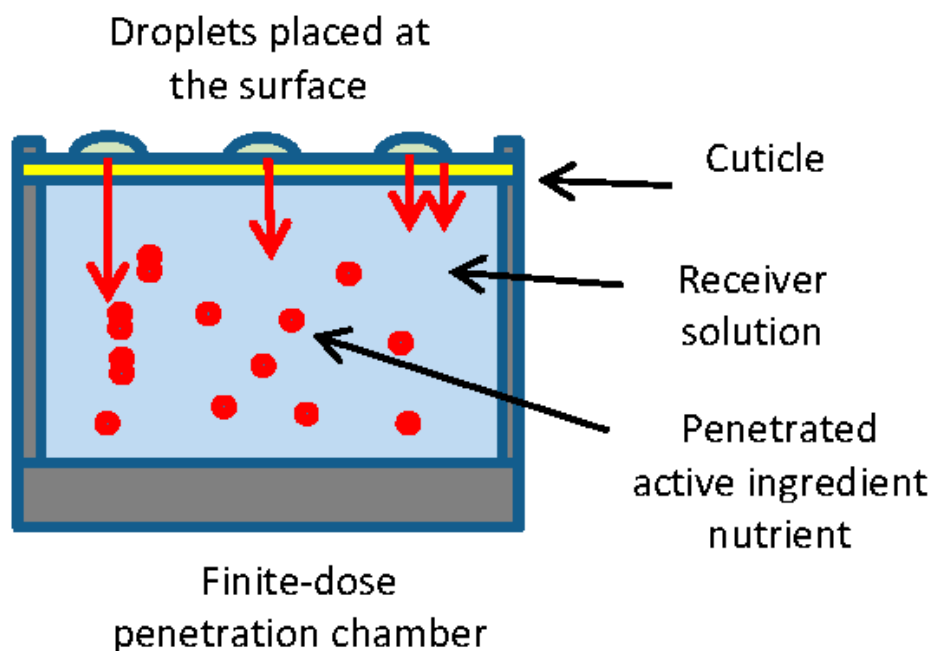
### 2.3. Surface Tension and Contact Angle

Surface tension (ST;  $n = 10$ ) was determined using the pendant drop method (IFT) and expressed in  $\text{mN} \cdot \text{m}^{-1}$ . The static contact angle (CA) was measured on both left and right-side of a sessile  $1 \mu\text{L}$  droplet placed on isolated tomato fruit cuticles ( $n = 10$  droplets). Both CA and ST were determined with a droplet shape analysis system (DSA 30E, Krüss GmbH, Hamburg, Germany).

### 2.4. Cuticular Penetration of Manganese and Zinc

The cuticular penetration was determined using the finite-dose system (Figure 1) by quantifying the amount of penetrated Mn or Zn after a predefined time. For this purpose, five  $1 \mu\text{L}$  droplets were gently deposited on the cuticles ( $n = 8$  for each treatment solution and cuticle type) with a Hamilton micro pipette (Hamilton Bonaduz AG, Hamilton, Switzerland). Immediately after application, the finite-dose penetration chambers were allocated inside a  $0.15 \text{ m}^3$  Perspex chamber which was kept under laboratory conditions.

The penetration time was between 46 h and 48 h. The average relative humidity was higher than 90%. After the penetration time, the cuticles were removed from the penetration chamber; the receiver solution was transferred to volumetric flasks (2 mL), which were filled up with distilled water. The Mn and Zn concentrations in the treatment groups and the reference to each treatment group ( $5 \times 1 \mu\text{L}$  solution droplets applied directly inside the 2 mL volumetric flasks) were analyzed by atomic absorption spectrometry (AAS, PerkinElmer, Analyst 300, Wellesley, MA, USA). The cuticular penetration was expressed as  $\text{mg} \cdot \text{L}^{-1}$  and percent (%) of the applied Mn or Zn.



**Figure 1.** Schematic representative of the penetration chamber. The stainless steel box is filled with deionized water as receiver solution which is in close contact to the mounted cuticular member (CM). The active ingredient (a.i.) of applied droplets penetrates through the CM [17].

### 2.5. Electron Microscopy

Characteristic micrographs of the dried deposits on leaves of tomato and paprika were generated with an environmental scanning electron microscope (ESEM, XL 30, FEI-Philips, Eindhoven, The Netherlands). For this purpose, droplets (1  $\mu\text{L}$ ) of the treatment solutions were applied to the adaxial side of the leaves and left to dry for two hours before analysis with the scanning electron microscope.

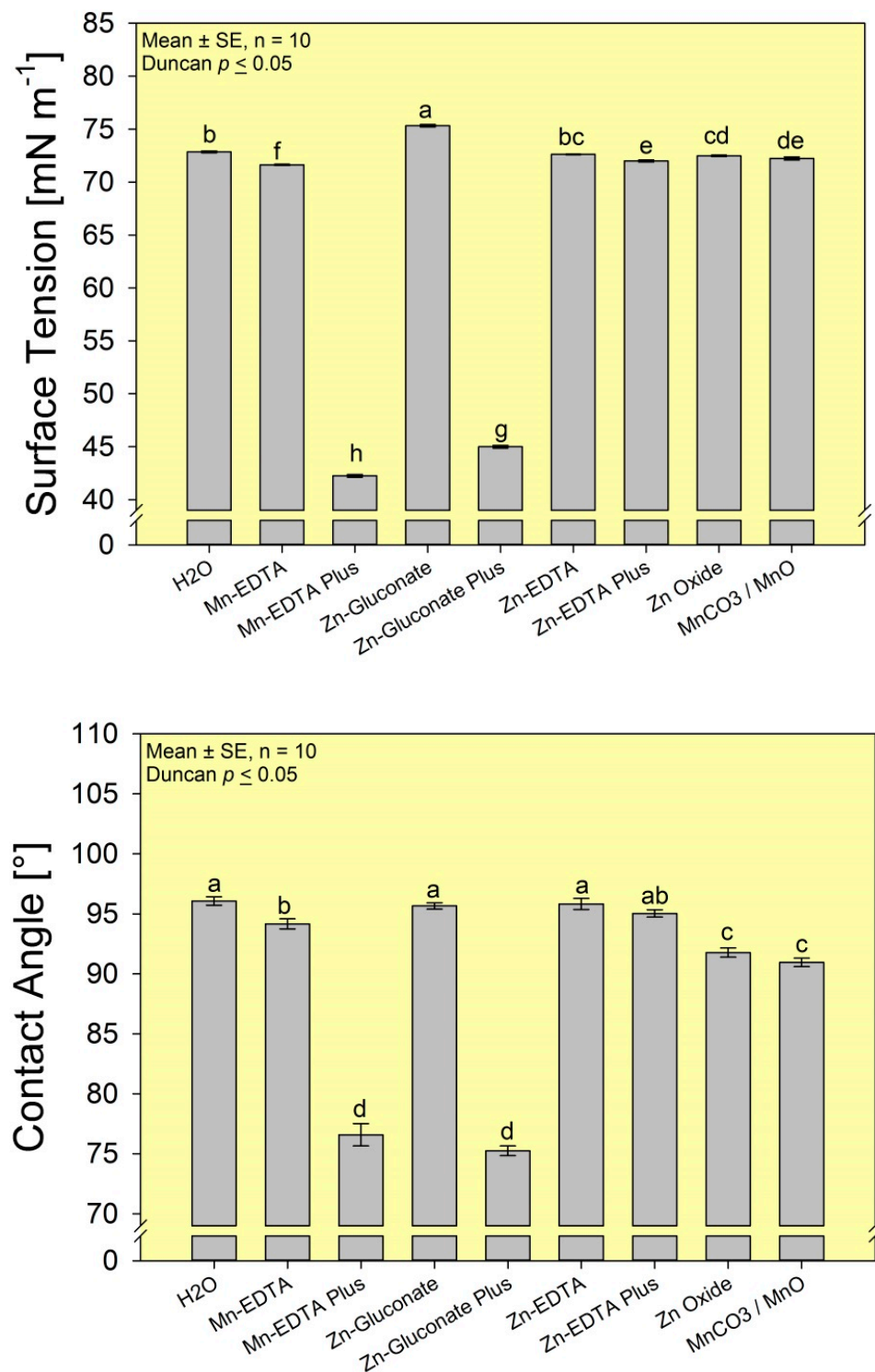
### 2.6. Statistics

Data were statistically analyzed with the software SPSS 20 (SPSS Inc., Chicago, IL, USA). Averages were compared by analysis of variance (ANOVA,  $p \leq 0.05$ ); when applicable, means ( $\pm$  SE) were separated by the Duncan multiple range test ( $p \leq 0.05$ ).

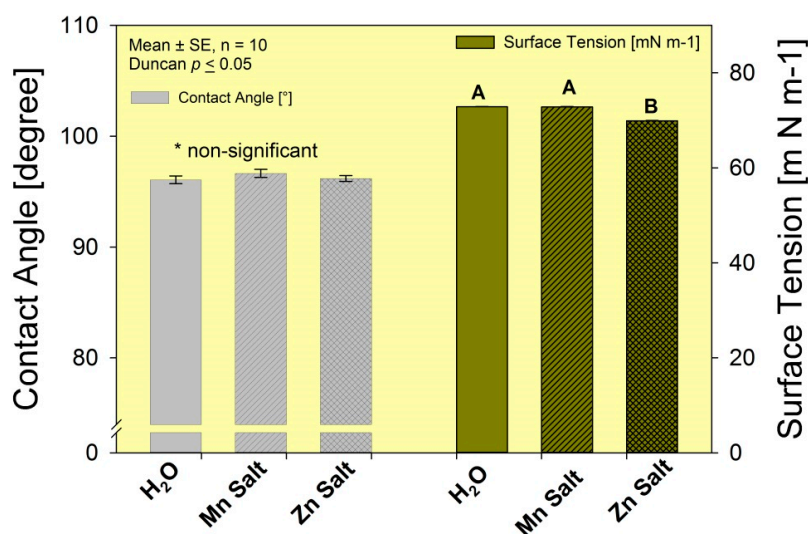
## 3. Results and Discussion

### 3.1. Surface Tension and Contact Angle

Surface tension (ST) of the liquids and contact angle (CA) of the sessile droplets strongly depended on the treatment solutions (Figures 2 and 3). As shown, the lowest surface tension was measured for Mn-EDTA Plus ( $\sim 42 \text{ mN} \cdot \text{m}^{-1}$ ) and Zn-Gluconate Plus ( $\sim 45 \text{ mN} \cdot \text{m}^{-1}$ ), followed by Mn-EDTA ( $\sim 70 \text{ mN} \cdot \text{m}^{-1}$ ). All other compounds had a slightly higher surface tension. In general, we observed the same trends for the contact angle, which was lowest for the 'Plus' formulations (both close to  $75^\circ$ ). Both ZnO and  $\text{MnCO}_3/\text{MnO}$  compounds showed a contact angle of about  $90^\circ$ , which is slightly lower than the other remaining formulations (close to  $95^\circ$ ).



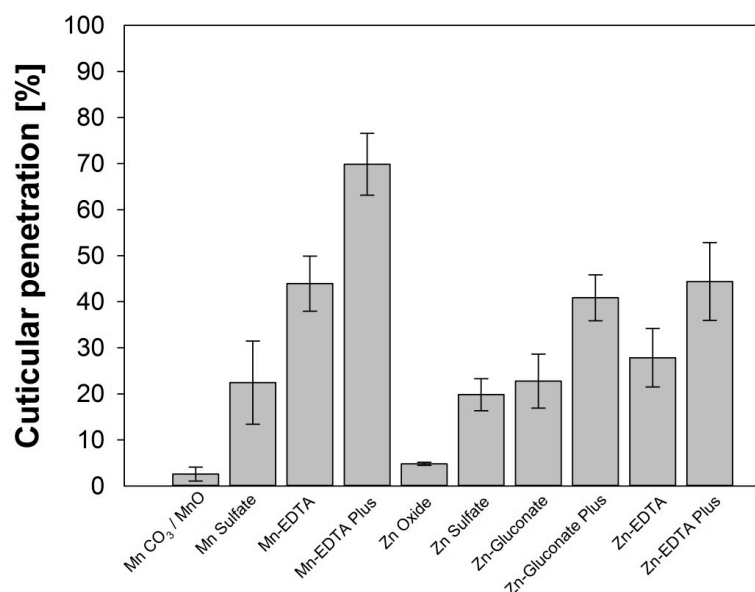
**Figure 2.** Surface tension and contact angle of the formulated suspensions. Values indicate the mean ( $\pm$  SE;  $n = 10$ ). Means were separated according to the Duncan multiple range test ( $p \leq 0.05$ ), values followed by different letters are statistically different.



**Figure 3.** Surface tension and contact angle of solutions of the non-formulated Mn- and Zn-sulfate salts. Values indicate the mean ( $\pm$ SE;  $n = 10$ ). Means were separated according to Duncan's multiple range test ( $p \leq 0.05$ ), values followed by different letters are statistically different.

### 3.2. Cuticular Penetration

The percent cuticular penetration is displayed in Figure 4. Considering the formulations containing Mn, the highest penetration was observed for Mn-EDTA Plus (~70% penetration) followed by Mn-EDTA (~44%). Considering the Zn-containing compounds, the highest percent penetration was observed for Zn-EDTA Plus (~46%). This is of particular interest, since Zn-EDTA Plus was applied at a significant lower dosage as compared to the other products. Considering all of the evaluated products, the ZnO and MnCO<sub>3</sub>/MnO formulations had the lowest cuticular penetration. Surprisingly the cuticular penetration of the Zn salt was very low (3.5%); similar results were observed for other compounds out of this study (Mauricio Hunsche, personal communication). As to the Mn salt, we observed a pronounced variation. On average, penetration from the Mn salt was about 22%.

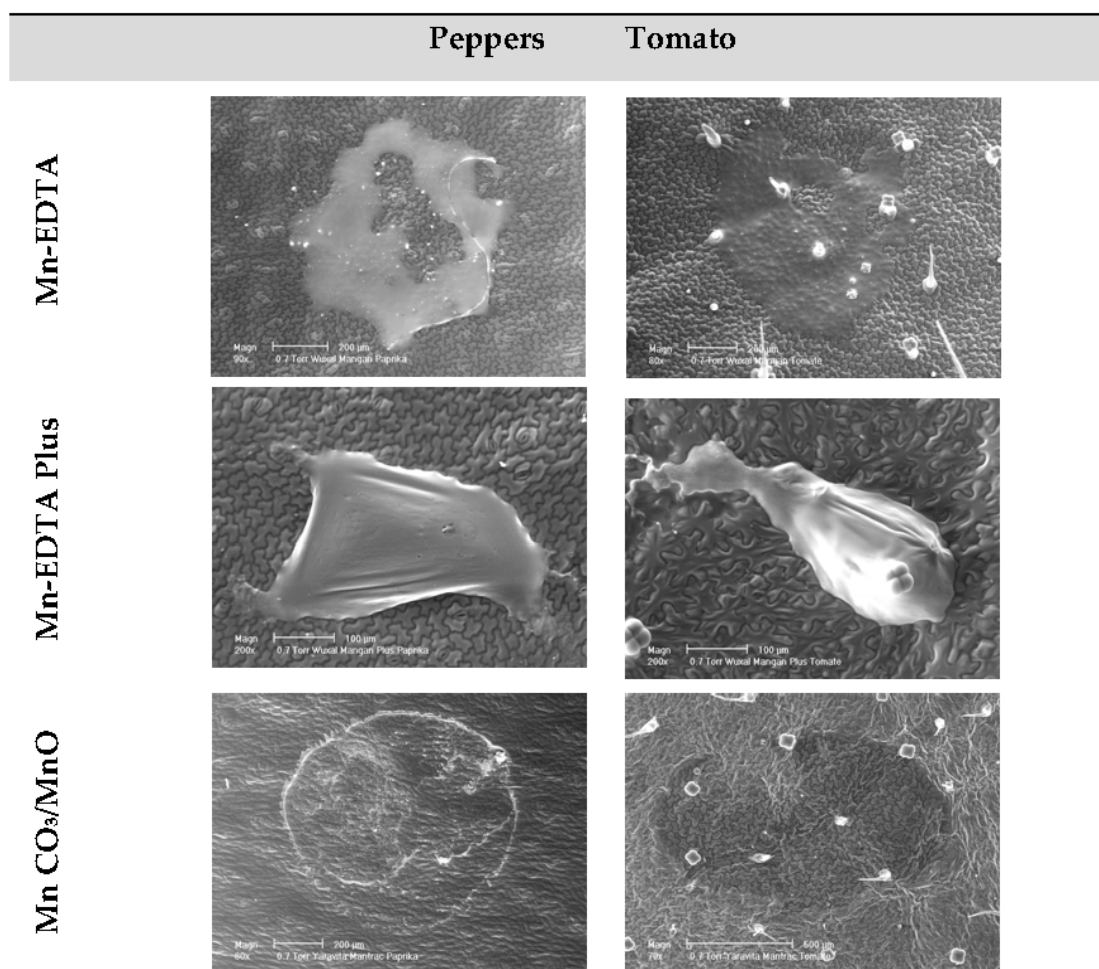


**Figure 4.** Cuticular penetration (%) of the selected compounds. Means  $\pm$  standard deviation ( $n = 8$ ).



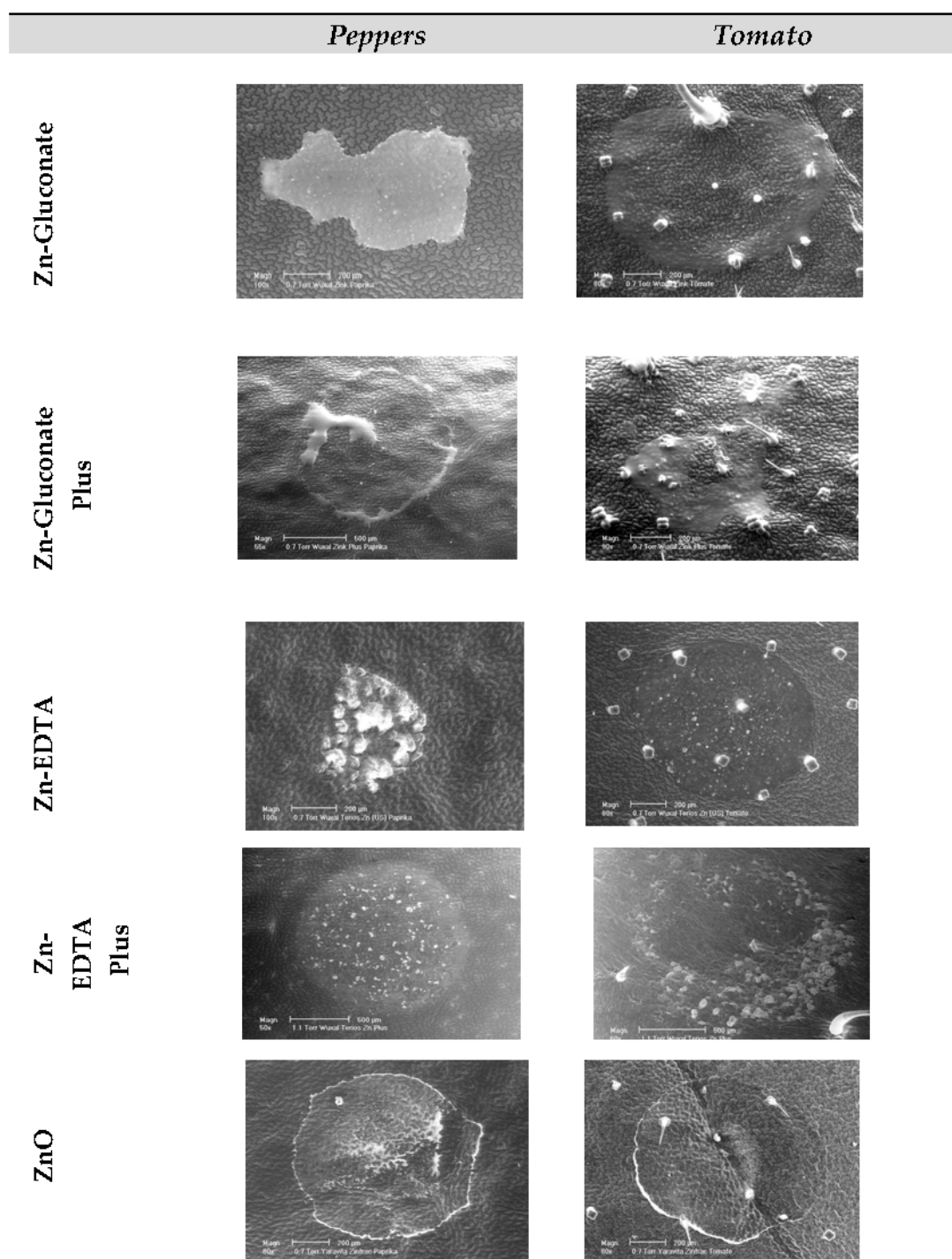
### 3.3. Deposit Properties: Scanning Electron Microscopy after Application of Formulated Products

Figure 5 shows representative deposits of the Mn-containing formulated products applied at the adaxial surface of peppers and tomato leaves. While the products Mn-EDTA and Mn-EDTA Plus formed rather concentrated and thick deposits, the  $\text{MnCO}_3/\text{MnO}$  product formed a deposit with more homogeneous distribution of dissolved/suspended particles leading, in many cases, to the occurrence of “coffee-ring” depositions, as previously observed for agrochemicals and adjuvants. Presumably, this small, but concentrated, deposit might have contributed for the higher cuticular penetration observed for Mn-EDTA Plus.



**Figure 5.** Representative micrographs taken with a scanning electron microscope (please note the scale of each figure). This picture table comprises the formulated manganese-based compounds used.

Figure 6 displays characteristic deposits of the formulated Zn-containing compounds when applied to the surface of peppers and tomato leaves. As shown, deposit characteristics were influenced by both treatment solutions and plant surfaces. In general, due to the shape and agglutination forms, deposits were easier to detect on the leaf surface of peppers. In case of Zn-Gluconate, Zn-Gluconate Plus, and Zn-EDTA easily identifiable deposits, often concentrated in smaller regions, were formed. Particularly for Zn-EDTA, fine crystalline structures were observed. Differently, Zn-EDTA Plus produced a more homogeneous deposit, whereas the ZnO-formulation produced a pronounced ‘coffee-ring’ like deposit.

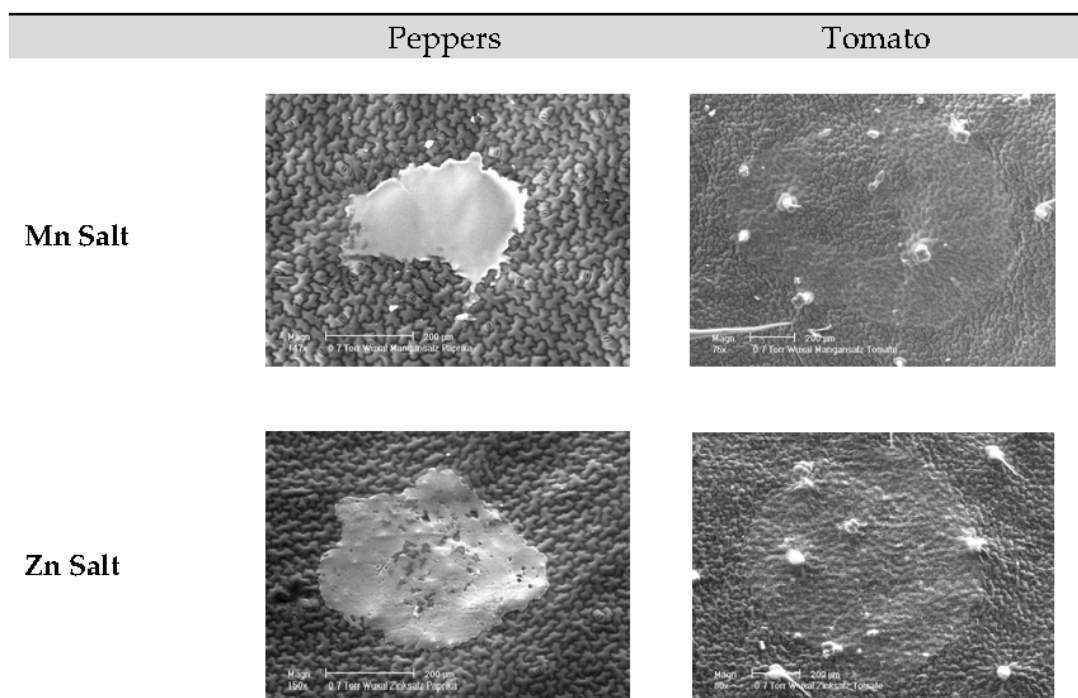


**Figure 6.** Representative micrographs taken with a scanning electron microscope (please note the scale of each figure). This picture table comprises the formulated zinc-based compounds used.

### 3.4. Deposit Analysis with Scanning Electron Microscopy after Application of Mn and Zn Salts

As shown in the SEM pictures (Figure 7), the deposit pattern and size strongly depend on the plant surface morphology. While on pepper leaves both Mn and Zn salts produced a thick crust, on tomato leaves the deposits had a larger area with homogeneous distribution of the dissolved particles over the whole deposit footprint.





**Figure 7.** Representative micrographs taken with a scanning electron microscope (please note the scale of each figure) used in Experiment 2.

#### 4. Conclusions

The experimental conditions used in this study (high relative humidity >90% and long penetration time) were selected to enable high cuticular penetration by all metal carriers involved. The use of a novel penetration enhancer significantly reduced the surface tension, as well as the contact angle, and the penetration rate of Mn could be considerably increased. In the case of Zn, this enhancing effect was remarkable in combination with the EDTA-chelated metal, but remained only slight with the gluconic acid-chelated Zn molecule. The comparative penetration rates of the Mn and Zn salt solutions also demonstrate that Zn ions penetrate at a distinctly slower rate through tomato cuticles than Mn ions. The reason for this difference is unknown, but might be due to specific cuticular interactions. The bio-efficacy of nutrient ions strongly depends on their penetration rate because uptake from the outer to the inner surface is the most limiting step. Compounds having higher penetration rates, as observed in this experiment, might enable higher biological efficacy under real field conditions.

The size and shape of deposits as analyzed with scanning electron microscopy strongly depends on the characteristics of the leaf surface. The formulations based on insoluble Mn and Zn compounds evidenced a very low cuticular penetration. This might be explained by their poor water solubility and, additionally, may be associated with the crystallization on the leaf surface. This is also documented by the SEM pictures.

An additional reason might be the size of these finely-ground particles. Mn carbonates and Zn oxides are usually available at a particle size of 20–100 microns, which is still too large for direct cuticular penetration. In leaves of plants, such as coffee or poplar, an average pore size of 4–5 nm was found [12], which would allow only the penetration of hydrated ions or even larger chelates reaching pore sizes between 0.5 to 1.5 nm.

An evaluation of a possible long-term penetration rate of these insoluble compounds through stomatous cuticular membranes or, alternatively, through the stomatal pathway was not within the scope of these experiments and should be investigated in further experiments.

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**Author Contributions:** A.A. prepared the experimental design and provided the experimental formulations as well as the penetration enhancer M.H. performed all the experiments and computed the data. A.A. and M.H. wrote the manuscript.

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