

Novel Chelating Agent for Trace Element Fertilizers

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INTRODUCTION

Worldwide, millions of hectares of arable land are deficient in plant available Zn and other trace elements such as Cu, Fe and Mn. On calcareous soils, adsorption and fixation reactions can substantially reduce fertilizer efficacy. Therefore, chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriamine pentaacetate (DTPA), are commonly used to increase the persistence of Zn and other trace elements in the soil solution. However, metal-EDTA and DTPA complexes are not readily absorbed by plant roots (Marschner 1995), which limits fertilizer efficacy. Zinc fertilizers could potentially be improved by using chelates that facilitate metal absorption by plant roots better.

Rhamnolipid is a biosurfactant produced by *Pseudomonas* bacteria. Rhamnolipid was identified as a novel Zn-complexing agent that may facilitate Zn absorption by plant roots via a novel lipophilic pathway and improve the efficacy of Zn and other trace element fertilizers.

METHODS

Octanol/Water Partition Coefficients to Measure Lipophilicity

Octanol/water partition coefficients ($K_{o/w}$) were determined using the shake-flask method. A series of 20ml solutions were prepared, containing 1mM ZnSO₄·7H₂O, CuSO₄·5H₂O and MnSO₄·4H₂O and rhamnolipid (mM) 0, 0.1, 0.24, 0.5, 1, 1.5, 2, and 2.5. Two ml of *n*-octanol were added to each vial before they were shaken end-over-end for 24 hours. Following shaking, 3ml of solution were removed from the lower 2cm of the water phase and digested in concentrated HNO₃ before analysis for total Cu, Mn and Zn by Inductively-Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The concentration of each metal partitioned in the octanol phase was determined by mass balance. Each treatment was replicated three times. The partition coefficient ($K_{o/w}$) was calculated by dividing the concentration of Cu, Mn or Zn in the octanol phase by their concentrations in the water phase (Chiou et al. 1977).

Rhamnolipid Application to Soil and Micronutrient Uptake by Durum Wheat

Ten durum wheat seeds (*Triticum durum*) were sown in plastic pots containing 1kg of clay soil (14% CaCO₃, pH 8.1). The soil was fertilized with a basal nutrient solution containing 200mg kg⁻¹ Ca(NO₃)₂, 100mg kg⁻¹ KH₂PO₄ and 20mg kg⁻¹ K₂SO₄. Zinc was applied as a ZnSO₄·7H₂O solution at 0.05 or 2 mg kg⁻¹ with 4 rates of rhamnolipid: 0, 0.75, 2, and 4 mg kg⁻¹. Each treatment was replicated three times.

Plants were grown in the glasshouse for 31 days before shoots were harvested, rinsed in de-ionised water, oven dried and weighed. Ground plant material (0.25g) was digested in concentrated HNO₃ and analysed by ICP-OES to determine Fe, Mn and Zn contents of the harvested shoots.

RESULTS AND DISCUSSION

Rhamnolipid formed lipophilic complexes with Cu, Mn, and Zn in fertilizer solutions (Fig 1). In rhamnolipid-free solutions, these ions were polar and excluded from the octanol phase. Rhamnolipid also significantly ($P \leq 0.05$) increased dry matter production and Fe, Mn and Zn uptake in durum wheat grown on calcareous soils (Table 1). Iron and Mn were not applied as fertilizers. Therefore, it appeared that rhamnolipid increased the plant-availability of the soil's native pool of these elements.

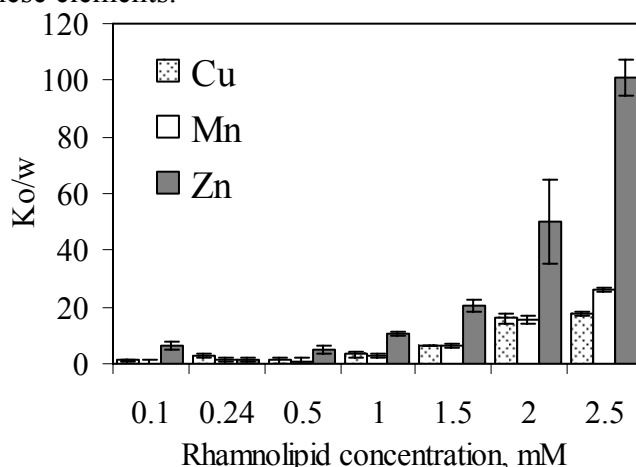


Fig. 1. Octanol water partition coefficients of Cu, Mn and Zn complexes with rhamnolipid.

Table 1. Fe, Mn and Zn contents of durum wheat shoots with four rhamnolipid application rates and 0.05 and 2 mg kg⁻¹ ZnSO₄ fertilizer. *

Rhamnolipid Application (mg kg ⁻¹)	Dry matter (g plant ⁻¹)	0.05 mg kg ⁻¹ Zn			2 mg kg ⁻¹ Zn			
		Fe (µg plant ⁻¹)	Mn (µg plant ⁻¹)	Zn (µg plant ⁻¹)	Dry matter (g plant ⁻¹)	Fe (µg plant ⁻¹)	Mn (µg plant ⁻¹)	Zn (µg plant ⁻¹)
0	0.12 ^a	16.08 ^a	24.64 ^a	1.40 ^a	0.24 ^a	16.36 ^a	20.58 ^a	8.02 ^a
0.75	0.14 ^b	17.95 ^{a,b}	30.50 ^{a,b}	1.42 ^a	0.27 ^{b,c}	16.93 ^{a,b}	25.66 ^{b,c}	8.95 ^{a,b}
2	0.16 ^c	19.86 ^b	32.60 ^b	1.90 ^a	0.26 ^c	17.00 ^{a,b}	23.78 ^{a,b}	9.36 ^b
4	0.17 ^c	22.84 ^b	35.63 ^b	1.68 ^a	0.29 ^c	18.50 ^b	25.30 ^{a,b,c}	10.62 ^c

*Same letters indicate that numbers within each column were not significantly different ($P > 0.05$).

CONCLUSIONS

Rhamnolipid formed lipophilic complexes with Cu, Mn and Zn cations. The published literature shows that lipophilic compounds may be readily absorbed by plant roots (Briggs et al. 1982, Phinney and Bruland 1994). Iron, Mn and Zn uptake in durum wheat may have been improved by the formation of these lipophilic complexes.

ACKNOWLEDGEMENTS

We would like to thank the Mosaic Fertilizer Company for funding and supporting this work.

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