

## Relationship Between Zinc Supply and Phosphorus Nutrition/ Phosphatase Enzyme Activity in a Hydroponically Grown Tomato Seedlings

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**Abstract:** Tomato (*Lycopersicon esculentum* Mill.) cultivar Moneymaker, was grown hydroponically in controlled temperature (C.T.) room for 25 days to investigate relationships between phosphorus concentration in leaves and roots with phosphatase enzyme activity in relation to zinc supply. Zinc was added at concentrations of 0.05, 0.5, 1 and 2 mg/L in complete nutrient solution. The 2 mg/L zinc treatment resulted in a reduction in dry weights compared with other treatments, but there were no significant differences in dry weights of seedlings between the other treatments. Zinc concentration in the plant increased with increasing zinc supply and reached a detrimental level with 2 mg/L Zn. However, there were significant decreases in P concentration in the leaves with increasing Zn supply and this decreased to an inadequate level in 2 mg/L Zn treatment. Phosphorus concentration in root increased with increasing Zn supply. Phosphatase enzyme activity was highest in the highest zinc treatment, with no significant differences in the activity in the other treatments.

**Key Words:** Tomato, zinc, phosphatase, hydroponic and phosphorus

### Su Kültüründe Yetiştirilen Domates (*Lycopersicon esculentum* Mill.) Fidelerinin Çinko Dozları ile Fosfor Beslenmesi/Fosfataz Enzim Aktivitesi Arasındaki İlişki

**Özet:** Moneymaker domates (*Lycopersicon esculentum* Mill.) çeşidi, çinko dozlarına bağlı olarak yapraklardaki ve kökteki fosforla fosfataz enzimi arasındaki ilişkiyi incelemek amacıyla 25 günlük süreyle kontrollü ısıtmalı odalarda yetiştirilmiştir. Besin çözeltisine 0.05, 0.5, 1 ve 2 mg/L dozunda çinko ilave edilmiştir. 2 mg/L uygulaması diğer uygulamalarla karşılaştırıldığında kuru ağırlıklarda azalmaya sebep olmuştur ancak kuru ağırlık bakımından diğer uygulamalar arasında önemli farklılık yoktur. Bitkideki çinko konsantrasyonu çinkonun artırılmasıyla artmış ve 2 mg/L çinko uygulamasıyla zararlı seviyeye çıkmıştır. Ancak yapraktaki P konsantrasyonu çinkonun artışıyla azalmış birlikte azalmalar olmuş ve fosfor, 2mg/L çinko uygulamasında yetersiz düzeye düşmüştür. Kökteki P seviyesi çinko konsantrasyonunun artmasıyla birlikte artmıştır. Fosfataz enzim aktivitesi en yüksek düzeye 2 mg/L çinko dozunda ulaşmış, ancak enzim aktivitesi diğer uygulamalarda önemli derecede değişmemiştir

**Anahtar Kelimeler:** Domates, çinko, fosfataz, su kültürü ve fosfor

#### Introduction

Phytotoxic amounts of metals are occasionally found in soils under natural conditions, but they originate more frequently from industrial and agricultural activities (Lepp, 1981). Stunted growth, discoloration, necrosis and deformation in leaf shape are obvious visible symptoms of severe metal toxicity (Woolhouse, 1983). Reduced dry matter production and nutrient imbalance is observed in crops grown in soils contaminated with moderate levels of heavy metals (Cottenie *et al.*, 1976).

The activity of some enzymes can increase significantly with high concentrations of heavy metals (Van Assche and Clijster, 1990). For example, it has been shown that tissues of some plants contain an increased phosphatase activity under phosphorus-deficient conditions (Lepp, 1981). This has been observed for tomato, barley, cucumber (Besford, 1979).

High level zinc in soil or nutrient solution can induce P deficiency. This may result in increased phosphatase enzyme activity in root of seedlings.

The aim of this short investigation was to find out relationship between phosphatase enzyme activity and phosphorus concentration in tomato seedlings grown at a range of zinc concentrations which included both optimum (0.5 mg/L) and high (2.0 mg/L) levels

#### Material and Method

This experiment was conducted at University of Hertfordshire in Bayfordbury Field Station in 1997. Seedlings of tomato cv., Moneymaker, were grown

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Table 1. Compositions of nutrient solution ( modified Hoagland and Arnon nutrient solution)

Salts used in nutrient solution	Elements	Concentrations (mg/l)
KNO <sub>3</sub>	K; N	195; 70
Ca(NO <sub>3</sub> ) <sub>2</sub> . 4H <sub>2</sub> O	Ca; N	200; 140
MgSO <sub>4</sub> . 7H <sub>2</sub> O	Mg; S	48; 64
KH <sub>2</sub> PO <sub>4</sub>	K; P	39; 31
H <sub>3</sub> BO <sub>3</sub>	B	0.5
MnCl <sub>2</sub> . 4H <sub>2</sub> O	Mn	0.5
CuSO <sub>4</sub> . 5H <sub>2</sub> O	Cu	0.02
H <sub>2</sub> MoO <sub>4</sub>	Mo	0.01
Na Fe EDTA. 1.5H <sub>2</sub> O	Fe	2.8
ZnSO <sub>4</sub> 7H <sub>2</sub> O	Zn	0.05

in complete nutrient solution in a growth chamber with relative humidity at 65-70% and the temperature at 23±2°C. The total photoperiod was 16 h/day and light intensity above the plant was between 20000-22000 Lux, depending on the exact height of the seedlings.

The composition of the complete nutrient solution used is shown in Table 1. This nutrient solution was modified by adding Fe in chelated form (Fe- EDTA) rather than as inorganic iron in order to prevent precipitation.

All solutions were prepared from "AnalaR" grade chemicals. The pH was adjusted to 5.6±0.2 immediately prior to use using a minimum volume of 0.1 M "AnalaR" KOH.

Seeds were germinated in glass beads moistened with 10% strength complete nutrient solution for a week. After germination seedlings were transplanted to a bowl filled with 10 L of continuously- aerated nutrient solution containing 0.5 mg/L zinc and held in place with cotton wool collars and grown on for a further week before receiving different zinc concentrations (0.05, 0.5, 1.0 and 2.0 mg/L) for 16 days. Nutrient solution was changed twice a week. Each treatment was replicated three times and each replicate included three seedlings (i.e nine seedlings per treatment).

Seedlings were grown on for five weeks, harvested, divided into component parts and dried at 70°C for 48 hours until constant weight was reached. Finally they were weighed to estimate dry weights.

#### Determination of phosphatase enzyme activity

In order to determine acid phosphatase activity in intact roots, the following procedure was followed:

The roots of three seedlings were transferred to beakers each containing 250ml nutrient solution plus the various concentrations of zinc (0.05, 0.5, 1.0 and 2.0 mg/L). A standard volume of P - nitrophenyl-phosphate (to give a concentration of 0.1 mM) was added to each beaker and pH adjusted to 4.0 with HCl. Control reactions were an aerated solution without seedlings. After 30 minutes 3.0 ml samples were drawn from each beaker and added to test tubes containing 1.0 ml of 2N NaOH. The tubes were shaken and centrifuged at 3000 g for 2 minutes and absorbance was read at 405 nm on a

spectrophotometer. Concentration of p- nitrophenol formed by the phosphatase mediated hydrolysis of p- nitrophenyl phosphate were determined by reference to standard curves of p- nitrophenol (Clark, 1975).

For the preparation of standards, 0.0070g product nitrophenol was weighed and made up to 50ml with deionised water to make solution 1000µM and 5ml of that solution was taken and made up to 100ml to make solution 50µM, finally 0, 1, 2, 3, 4, 5 and 6 ml of 50µM solution were taken and made up to 50 ml with deionised water to prepare 0, 1, 2, 3, 4, 5 and 6µM solution.

#### Chemical analyses

Chemical analyses were carried out on plant material dried at 70°C for 48 hours, ground to powder using a pestle and mortar and stored in polyethylene bottles. Samples of powdered plant material, 0.2 - 0.5 g dry weight, were dry ashed at 550°C in a muffle furnace prior to analysis. The white ash was taken up in 2 M hot Analar HCl, filtered and diluted to 50 ml with deionised water.

Samples were analysed for Zn (Unicam Solar 929 A.A.S.) with an air acetylene flame. P was analysed by a vanadate molybdate method using a Pye Unicam SP6-550 UV/Vis. Spectrophotometer (Chapman and Pratt, 1982).

#### Statistical analyses

The differences in plant dry weight and the concentrations of nutrients within the plant from the various zinc treatments were statistically tested for significance using an Statview-ANOVA test ( p< 0.05). Statistically different groups were determined by LSD test.

#### Results and Discussion

##### Plant growth

Table 2 shows that both the total and shoot dry weight of tomato plant decreased significantly (p>0.05) in the highest (2 mg/L) Zn treatment. Although the highest

shoot and total biomass was shown by seedlings in the 0.5 mg/L Zn treatment there were no significant differences in shoot and total dry weights between zinc treatments ranging from 0.05 to 1 mg/L. Root dry matter values in both the 2 and 1 mg/L Zn treatments were the same, both showing a significant reduction compared. These results are in agreement with Tomar *et al.* (1994) who reported that in a pot experiment with sunflower plants subjected to high levels of zinc (10 mg/kg soil) had reductions in the dry matter.

Visible effects in seedlings receiving zinc at 2 mg/L began to appear about 2 weeks after exposure to Zn at this high level. This zinc concentration inhibited both roots and shoot growth of tomato plant. Tomato seedlings showed symptoms of zinc toxicity, roots became brown in colour, and the growth was limited and leaf size was smaller than normal with chlorotic appearance.

#### Zinc concentration within plant

The data in Table 3 show that zinc concentration of all parts of the plant increased significantly with increasing levels of zinc and reached a toxic level in 2 mg/L Zn treatment; a value higher than 250 µg/g is reported to be toxic for tomato seedlings by Adams (1986). These results are in agreement with those reported by Ruano *et al.* (1988) in bush bean seedlings, by Parker *et al.* (1992) in tomato plants and by Loneragan *et al.* (1982) in okra plants.

Zinc concentrations were at adequate levels in leaves of seedlings in the other treatments. There were vast increases in the root Zn of seedlings grown at 2 mg/L. This is probably due to hyper-accumulation of micro-nutrient in root as a stress response created by high concentration of zinc in growth medium.

#### Phosphorus concentration within plant

Table 3 also shows that the phosphorus concentration was at inadequate levels in the leaves of the plant in 2 mg/L Zn treatment. A value of P less than 0.3 % for tomato was reported (Tomar *et al.*, 1994) to be deficient. The results of the phosphorus concentrations in seedlings grown at high zinc are in agreement with those reported by Loneragan *et al.* (1982) who noted that toxic levels of zinc strongly depressed phosphorus concentration in leaves of okra seedlings.

In this experiment, P concentration was significantly higher in the roots of plant in 2 mg/L Zn treatment than that of the other treatments. This may be because the high zinc concentration interferes with translocation of P from roots to shoot resulting in P accumulates in root tissue. There was negative correlation ( $r=-0.94$ ) between zinc and phosphorus in the leaves.

Table 2. Dry weight of shoot, roots and total dry matter of seedlings grown at different levels of zinc (g/plant)\*

Zn conc. (mg/L)	Shoot	Root	Whole plant
0.05	0.72 b	0.09 b	0.81 b
0.50	0.78 b	0.09 b	0.87 b
1.00	0.72 b	0.06 a	0.78 b
2.00	0.60 a	0.06 a	0.66 a

\*Means of three replicates and each replicate includes two seedlings  
Within each column, same letter indicates no significant differences between zinc treatments ( $P > 0.05$ )

Table 3. Zinc and Phosphorus concentration of the leaf and root tomato seedlings grown at different levels of zinc (on dry weight basis)\*

Zn conc. (mg/L)	Zinc (mg/kg)		Phosphorus (%)	
	Leaf	Root	Leaf	Root
0.05	43 a	118 a	0.78 c	1.18 a
0.50	73 b	523 b	0.72 c	1.24 ab
1.00	164 c	1512 c	0.55 b	1.45 b
2.00	255 d	3605 d	0.25 a	2.05 c

\*Means of three replicates and each replicate includes two seedlings  
Within each column, same letter indicates no significant differences between zinc treatments ( $P > 0.05$ )

Table 4. Phosphatase enzyme activity (µM PNP/ g dry weight root/h)\* in tomato seedlings grown at various levels of zinc

Zinc conc. (mg/L)	µM PNP/ g dry weight root/h (enzyme activity)
0.05	86.6 a
0.5	98.6 a
1.0	105.8 a
2.0	185.8 b

\*Means of three replicates and each replicate includes three seedlings  
PNP: P-nitrophenyl phosphate  
Within each column, same letter indicates no significant differences between zinc treatments ( $P > 0.05$ )

#### Phosphatase enzyme activity

Table 4 clearly indicates that the acid phosphatase activity in intact roots of tomato seedlings was increased with increasing zinc supply and reached its highest level in the high (2.0 mg/L) Zn treatment where the seedlings showed evidence of both Zn toxicity and P deficiency in the leaves. Increases in the acid phosphatase activity in the roots of seedlings in 2.0 mg/L may be associated with low P concentration in the leaves. Bielski (1973); Maclachan (1976) reported that phosphatase activity would increase in the root where phosphorus supply is inadequate.

Positive correlation ( $r = 0.86$ ) was found between zinc supply and phosphatase enzyme activity and this was statistically ( $P < 0.05$ ) significant.

### Conclusion

In the light of this experiment, it can be concluded that:

- 1) 0.05 to 1 mg/L Zn range may be considered to be adequate/optimal.
- 2) 2 mg/L zinc was the detrimental level to the plant growth
- 3) 2 mg/L zinc treatment resulted in both zinc toxicity and phosphorus deficiency in seedlings of tomato cv., Moneymaker
- 4) Phosphatase enzyme activity increased in roots of seedlings exhibiting phosphorus deficiency and zinc toxicity (i.e. in the 2 mg/L Zn treatment).

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