Asian Journal of Chemistry Vol. 22, No. 9 (2010), 6991-6997

Manganese Toxicity on Manganese Accumulation and Mineral Composition of Tomato (*Solanum lycopersicum* L.)

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A greenhouse pot culture experiment was conducted to determine the effects of increasing manganese dose (0, 50, 100 or 200 mg L^{-1} Mn) on growth, manganese accumulation and element changes in tomato (*Solanum lycopersicum* L. cv. Falcon) seedlings. Excess Mn as 100 and 200 mg Mn L^{-1} decreased all the growth parameters according to control plants. Manganese accumulation significantly increased in the parts of the seedlings with increasing amounts of Mn. A positive interaction was observed between 50 mg L^{-1} Mn and uptake of all elements except Zn content in root, shoots and leafs and Ca content in leaf of in seedlings and growth. Elevated Mn as 100 and 200 mg L^{-1} were commonly limited growth and uptake macro-micro elements by plant parts. Effects of Mn in low concentrations as 50 mg L^{-1} dose of Mn was stimulate but Mn in excess levels as 100 and 200 mg L^{-1} was limited for tomato seedlings characteristics.

Key Words: Manganese, Toxicity, Tomato.

INTRODUCTION

Manganese is a trace element essential for living organisms include plants as a nutrient participates in photosynthesis, enzyme activation, carbohydrate metabolism and phosphorylation[1]. It also functions in chloroplasts as part of electron transfer (oxidation-reduction) reactions and electron transport systems[2].

Cultivation of crop species and cultivars resistant to high soil manganese may reduce with the negative effects of Mn toxicity. Manganese of some acid soils can be as high as 50 mg kg^{-1} in soil solution and toxicity problems may be present in crops such as beans, cereals, maize and tomato[3]. Leaf concentrations above 300 μg g^{-1} Mn may be toxic[2]. The symptoms of Mn toxicity is necrotic tissue along the main vein of the leaf blades is bordered by yellow chlorotic areas[4]. Related to damages, soils with toxic level of manganese are appeared in Mn toxicity is one of the limiting factors for germination, root and plant growth-development, biomass and finally crop yield in agricultural cultivation[5]. Furthermore, excess Mn in the medium can interfere with the plant's absorption, translocation and utilization of

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other mineral elements such as iron, calcium and magnesium, thus confounding the primary cause of Mn toxicity symptoms in some plants[1,6,7]. But still, there are inadequate research on relationships the manganese toxicity with other macronutrient and micronutrient in many plant species as a tomato.

These investigation objectives were determine application of external excess of Mn on (1) manganese and other macro and microelements uptake and accumulation in root, shoot and leaf, (2) on growth and biomass, (3) effects of manganese toxicity in tomato seedlings.

EXPERIMENTAL

Seeds of tomato (*Solanum lycopersicum* L. cv. Falcon) were germinated and cultivated in pots (600 cm^3) containing standard soil:lamb manure (3:1) mixture in natural light, mean air temperature of 25/15 ± 3 °C (day/night), relative air humidity of 75 ± 10 %, in a greenhouse. Each pot or seedling was watered every 2 days with tap water (100 cm^3) and 30 days after germination. Then, Mn was added every 4 days and 20 days (5 times) to the pots as 0 (control), 50 (low), 100 (moderate) and 200 (high) mg L^{-1} of Mn (as $MnSO_4$) with 100 cm^3 water as experimental treatment. The experiment was arranged in a completely randomized blocks design. Each treatment replicated three times and each replicate included 25 plants.

The leafs, shoots and roots of plant were washed by tap and deionized water two times and dried at 60 ºC for 48 h. The dried tissues were weighed and ground into a fine powder before wet ashing in $HClO_4$:HNO_3 (4:1, v:v) solution. Calcium, magnesium, potassium, phosphorus, iron, zinc, copper, sodium and manganese of plant parts were determined by atomic absorption spectrophotometer (Perkin-Elmer 3100, USA).

The analysis of variance for all the parameters was computed on statistically significant differences determined based on the appropriate F-tests. Differences between the mean values were compared utilizing Duncan test.

RESULTS AND DISCUSSION

Plant growth and biomass: Tomato seedling growth and biomass were significantly depressed by the Mn concentrations (Table-1). Root, shoot and leaf growth were generally enhanced at low Mn concentration (50 mg L^{-1} Mn) but limited at moderate (100 mg L^{-1} Mn) and high Mn dose (200 mg L^{-1} Mn). Similarly root, shoot and leaf biomass increased in 50 mg L^{-1} and then decreased with increased Mn from 100-200 mg L^{-1}.

Manganese uptake and distribution: Low level of manganese concentrations (50 mg L^{-1} Mn) increased Mn contents of the tissues of tomato seedlings. Exposure to excess Mn, particularly 100 and 200 mg L^{-1} of Mn, led to more increased accumulation of Mn in root, shoot and leaf. At the increase Mn concentrations, leafs accumulated more Mn than the shoots and roots (Fig. 1).

TABLE-1
LENGTH AND BIOMASS IN PARTS OF TOMATO SEEDLINGS AS AFFECTED BY DIFFERENT MANGANESE CONCENTRATIONS. VALUES ARE MEANS (n = 3)

Manganese (mg L^{-1})	Root		Shoot		Leafs	
	Length (cm)	Dry weight (g)	Height (cm)	Dry weight (g)	Area (cm^2)	Dry weight (g)
0	14.30ab	0.47ab	30.70b	2.25b	174.9a	1.86a
50	15.74a	0.53a	34.00a	2.48a	180.3a	2.02a
100	12.63b	0.33bc	29.60b	1.95c	113.8b	1.75a
200	9.95c	0.21c	22.30c	1.34d	90.9b	1.05b
p	< 0.01	< 0.05	< 0.001	< 0.001	< 0.01	< 0.01
$LSD_{0.05}$	2.07	0.17	3.06	0.15	45.12	0.38

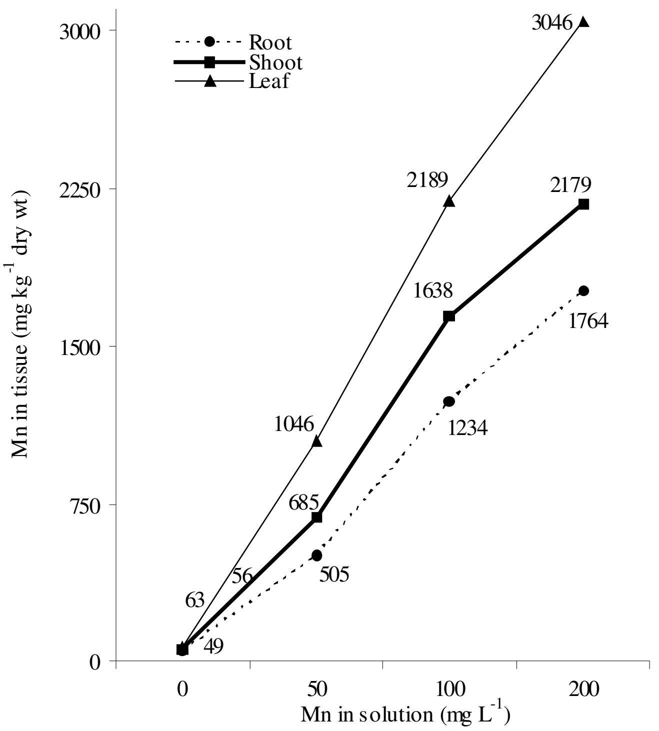

Fig. 1. Manganese concentration in various tissues of tomato seedling as a function of Mn concentration in solution (n = 3). Mn content in root, shoot, and leaf is significant at $p < 0.001$. $LSD_{0.05}$ values are 246.36, 380.08 and 723.22 in roots, shoots and leafs, respectively

Macro, microelements and sodium uptake and distribution: Analysis of variance (not shown) indicated that macroelement, microelement and sodium accumulation was affected by excess of Mn concentrations in solution (Table-2). Calcium content in root and shoot increased at 50 mg L^{-1} of Mn and then decreased at 100 and 200 mg L^{-1} Mn, which brought the root and shoot Ca content in line with the no-Mn treatments. Leaf Ca content was progressively decreased with increasing Mn concentrations. Magnesium content in shoots and leafs was significantly changed with the increasing Mn concentrations, but insignificant in roots. Roots in different Mn concentrations were accumulated more Mg than the shoot and leafs. Excess manganese were only affected potassium contents in root. Potassium content of shoots and leafs was insignificant but still potassium content in these tissues increased in low Mn concentration, but decreased in moderate and high Mn concentrations. Although there were some exceptions such as leaf phosphorus content in 100 mg L^{-1} of Mn, the content of P in roots, shoots and leafs increased at Mn level, but decreased with in moderate and high Mn concentrations. The P contents of leaf was more than roots and shoots. On increasing manganese concentrations, iron contents were significantly affected the root and leaf, but Fe content was insignificant in shoot. Roots were accumulated more Fe than leafs and shoots. Manganese concentrations were significantly declined zinc contents of shoot and leaf. But root Zn content was insignificant in Mn concentrations. Although Zn content of root was not significant, the distribution of Zn within the plant followed the trend leafs > shoots > roots. While 50 mg L^{-1} of Mn increased the amount of copper uptake into the roots and leafs, the effect decreased with 100 and 200 mg L^{-1} of Mn. Copper contents of shoot did not have a significant effect up to Mn concentrations. With Mn level increased, the distribution of Cu was leafs ≥ roots > shoots. Sodium accumulation in tomato roots, where low Mn concentration had enhancing effect, while excess Mn depressed uptake. Moreover, Na uptake was increased in low manganese but Na uptake clearly depressed by increasing Mn treatment in shoot and leafs. Roots were Na content in roots than sodium content of shoots and leafs.

Moreover, Table-3 shows the some linear correlations which can be significant among manganese content with different macroelements, microelements and Na for the root, shoot and leaf of tomato seedlings. Concentrations of Ca were negatively related with the Mn concentration in roots, shoots and leafs. Content of Mg was only correlated with Mn content in shoots ($r = -0.76$, $p < 0.05$). A correlation were insignificant between Mg and Mn contents in roots and leafs. The contents of K in leaf ($r = -0.61$, $p < 0.05$) and shoots ($r = -0.77$, $p < 0.01$) of tomato seedling were related with the Mn contents. Any correlation between K and Mn contents in leafs was not found. Non-correlation was obtained contents of P with the Mn contents in tissues. Fe concentration was negatively correlated with the Mn content in roots, shoots and leafs $r = -0.62$, -0.58 and -0.60 at $p < 0.05$, respectively. Correlations between Zn and Mn were $r = -0.64$ ($p < 0.05$), $r = -0.76$ ($p < 0.01$) and $r = -0.77$ ($p < 0.01$) in roots, shoots and leafs, respectively. The concentrations of Cu in leaf ($r = -0.62$, $p < 0.05$) and roots ($r = -0.55$, $p < 0.05$) of tomato seedling were related with the Mn concentrations. Any correlation was not found in shoots. Na concentration decreased with increasing Mn concentration in leafs and roots with $r = -0.74$ and $r = -0.73$ at the $p < 0.05$, respectively. Na content was not related with the Mn content in shoots.

TABLE-2
MACROELEMENTS AND MICROELEMENTS, AND SODIUM CONTENT IN PARTS OF TOMATO SEEDLINGS AS AFFECTED BY DIFFERENT MANGANESE CONCENTRATIONS. VALUES ARE MEANS (n = 3)

Manganese (mg L^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	P (mg g^{-1})	Na (mg g^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
				Roots				
0	15.7	9.3	10.8	2.5	4.4	156.8	38.2	11.2
50	16.8	10.2	16.4	7.6	4.5	162.0	30.1	13.2
100	14.8	11.3	17.1	4.8	3.8	110.2	27.3	10.5
200	10.8	10.7	16.6	4.6	2.9	78.4	20.9	6.6
p	< 0.001	ns	< 0.001	< 0.001	< 0.01	< 0.01	ns	< 0.01
$LSD_{0.05}$	0.93	1.61	1.01	0.45	0.62	41.48	11.93	2.63
				Shoots				
0	23.3	8.7	22.8	1.5	2.7	14.8	57.7	4.0
50	33.6	9.6	23.8	4.9	4.2	17.7	32.5	5.9
100	20.5	8.1	17.8	3.1	2.8	10.3	27.9	5.3
200	12.6	7.0	16.2	2.9	2.4	5.7	25.5	3.6
p	< 0.001	< 0.01	ns	< 0.001	< 0.001	ns	< 0.001	ns
$LSD_{0.05}$	3.91	0.86	6.60	0.23	0.52	8.57	7.85	1.77
				Leafs				
0	33.6	7.0	18.7	4.6	1.2	30.1	102.9	13.8
50	28.0	7.1	23.5	4.8	1.3	35.3	40.7	15.1
100	26.8	6.8	21.7	5.5	0.8	28.9	38.7	11.2
200	24.6	5.4	21.4	4.6	0.6	14.0	32.8	10.0
p	< 0.05	< 0.001	ns	< 0.05	< 0.01	< 0.01	< 0.001	< 0.01
$LSD_{0.05}$	4.46	0.43	6.35	0.55	0.23	6.9	12.02	1.97

TABLE-3
CORRELATIONS OF MANGANESE CONTENT WITH MINERAL ELEMENTS IN DIFFERENT PARTS OF TOMATO SEEDLINGS; n = 10. (^{ns}, * AND **: INSIGNIFICANT AND SIGNIFICANT AT p < 0.05 AND p < 0.01, RESPECTIVELY)

				Content in tissue				
Mn	Ca	Mg	K	P	Na	Fe	Zn	Cu
Root	-0.683*	0.470^{ns}	0.731**	0.153^{ns}	-0.750**	-0.634*	-0.633*	-0.576^{ns}
Shoot	-0.671*	-0.717*	-0.614*	0.149^{ns}	-0.361^{ns}	-0.591*	-0.792**	-0.145^{ns}
Leaf	-0.660*	-0.523^{ns}	0.295^{ns}	0.344^{ns}	-0.740**	-0.604*	-0.774**	-0.623*

High concentration of Mn found in the tissues especially leafs in comparison to the control, correlated to the increase of the content of the metal. These findings are in agreement with the observations of previous researches in wheat, cowpea and tobacco[8-11]. Also, in this study, the distribution of Mn was leafs > shoots > roots in increased Mn concentrations. The highest concentrations of Mn in the leafs were indicated the transport of Mn *via* the xylem sap in present investigation. This tolerance mechanism could partly explain the higher Mn accumulation in the foliage compared with the tomato shoots and roots. This finding is consistent with cowpea[12], rice, pumpkin[13] and sweetpotato[14]. Low-electronegative metals, such as Mn, are translocated easily to the foliage of plants[14]. This result may explain the alterations of chloroplasts described as *in vitro* tobacco plants, treated with excess Mn. In these plants spongy mesophyll cells is showed a reduced number of chloroplasts with disrupted grana and rich in plastoglobuli and proteic bodies. At normal concentration, Mn plays a role in protecting chlorophyll from photooxidation. Therefore, it is possible that the presence of high concentration of Mn in the leafs causes oxidation of either chlorophyll or chloroplast membranes, in addition to other effects at photosynthetic levels[1,9,11].

Manganese affected other elements content in tissue of tomato seedlings. All elements accumulation in tomato roots, shoots and leafs where low Mn concentration as 50 mg L^{-1} had enhancing effect, while excess Mn as 100 and 200 mg L^{-1} dose depressed uptake. These results confirmed for some elements but no relationships was found between Mn and other elements. Osawa and Ikeda[8] used top dry weights to evaluate relative tolerance to manganese (0.5, 3.0, 10.0, 30.0 and 100.0 ppm) in 14 species of vegetable seedlings. They obtained that effect of excessive manganese on the iron concentration in leafs varied the species. Calcium and magnesium in leafs in some species was decreased by the excess of manganese. But they defined that the concentrations of copper, zinc, phosphorus and potassium in leafs had no relationship with the manganese toxicity.

Symptoms of manganese toxicity in various species showed considerable variety. Brown necrotic spots and chlorosis were observed in many species. Brown necrotic spots occurred in the marginal area of older leafs or along the lower portion of stems. At least part of the chlorosis resembled iron deficiency symptoms. Excess Mn induced no serious abnormality in roots except a brownish discolouration in some species[8].

Excess Mn accumulation and imbalance of elements in tissues caused to growth and biomass depression for tomato seedlings. These results are parallel with peas that Mn toxicity limited plant growth and biomass[15]. Nevertheless, although high Mn concentrations found in the tissues increasing Mn levels (Mn content of leaf reached 1046 μg g^{-1} in 50 mg Mn L^{-1}), characteristic toxicity symptoms were not observed in plants except 200 mg Mn L^{-1} in solution. This case may be dangerous for human who are fed with tomato. Because, normal range is between 24 and 35 μg g^{-1}, above 300 μg g^{-1} Mn in leaf may be toxic in tomato[4].

Conclusion

Elevated manganese concentrations were negatively affected the root, shoot and leaf growth and biomass; manganese accumulation and other element contents in root, shoot and leafs. Usually growth and biomass and element contents increased in 50 mg L^{-1} but decreased with Mn from 100-200 mg L^{-1}.

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(*Received*: 7 January 2010; *Accepted*: 14 June 2010) AJC-8790