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Interaction Effects of Zinc and Manganese on Growth, Uptake Response and Chlorophyll Content of Sweet Corn (*Zea mays* var. *saccharata*)

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Abstract: Manganese (Mn) and Zinc (Zn) interact with each other and this interaction can result in impacts on the yield of corn plants. This study was conducted to examine the effect of different levels of Mn and Zn on the yield, Mn and Zn concentration, root growth parameters and chlorophyll contents of corn plants. Sweet corn was grown in nutrient culture containing all combinations of Zn and Mn at levels of 0.0, 0.1, 1.0 and 10.0 mg L^{-1} as $ZnSO_4.7H_2O$ and $MnSO_4.H_2O$, respectively and harvested at 28 days after transplanting. Mn and Zn concentrations in roots and shoots increased with increasing Mn and Zn concentration in nutrient solution. Zn concentration in both roots and shoots enhanced with increasing Mn levels. Mn concentration in shoots did not show any correlation with Zn concentration in nutrient solution, but Mn concentration in roots decreased with increasing levels of Zn. Zn_0Mn_1 treatment produced the highest yield. The lowest dry weight of young corn plants was recorded under $Zn_{10}Mn_0$ treatment due to Mn deficiency. Chlorophyll content decreased with high Zn application and this can be attributed to the interaction of Zn with iron in the growth medium. Different levels of Zn and Mn in nutrient solution did not have any significant effect on root parameters.

Key words: Zinc, manganese, corn, concentration, chlorophyll

INTRODUCTION

Manganese (Mn) and Zinc (Zn) are two essential micronutrients for optimum plant growth and a deficiency of just one of them can cause a considerable reduction of yield. Zn is a metal component of over 300 enzymes in plants (Brown *et al*., 1993; Welch and Shuman, 1995), required for photosynthesis and sugar formation, protein synthesis and the maintenance of membrane integrity (Lopez-Millan *et al*., 2005). Mn is required for the activity of enzymes (Welch and Shuman, 1995; El-Jaoual and Cox, 1998; Ducic and Polle, 2007), oxygen evolution in photosynthesis (Fox and Guerinot, 1998), detoxification of oxygen-free radicals, CO_2 fixation (Welch and Shuman, 1995; Fox and Guerinot, 1998), auxin catabolism (Marsh *et al*., 1989), ribosome structure and disease resistance (Welch and Shuman, 1995). Compared with other transition metals, Mn transport is efficient within the plants. It can be transported by many pathways (Hall and Williams, 2003; Pittman, 2005), absorbed independently with other micronutrients (Bowen, 1969) and is rather coordinated with oxygen donors (Brown, 1963). While Zn moves as an anion in the form of Zn-citrate or malate, Mn moves as a cation in plants (Tiffin, 1967; Grusak *et al*., 1999). In addition, while Zn is considered as the most mobile micronutrient, Mn is not easily remobilized in plants (Grusak *et al*., 1999).

An adequate supply of Mn and Zn for plants depends on many factors such as the parent rocks which the soils are derived (Wijebandara, 2007), soil factors such as pH and the level of clay and organic matter, rate of absorption by plants and rate of translocation within the plant. An interaction occurs when the level of one nutrient influences the other in relation to plant growth (Olsen, 1972). Interaction between two nutrients may take place in the soil or within the plant. A nutrient may reduce the translocation rate of the other nutrient or may cause the enhancement of the yield and decrease the concentration of the other nutrient (dilution effect) or may reduce the uptake of the other nutrient at the site of absorption by the roots.

Yoshiaki and Ando (1968) demonstrated that the growth of the rice plants depends on both Zn and Mn concentration in tissues and the ratio of Mn to Zn in the tissues. High yield can be obtained even at high Zn and Mn concentration in tissues, if the Mn/Zn ratio in tissues is in the range of 0.1-10. They showed that Mn level could highly affect the critical Zn concentration resulting in Zn

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toxicity symptoms. Barben *et al.* (2010a, b) observed that Mn concentration in potato tissues decreased with increasing Zn concentration in nutrient solution from deficient to optimal and then increased as available Zn enhanced from optimal to excessive.

The effect of available Mn on Zn concentration in plants and the correlation between Zn and Mn in plants is rarely studied. This study aimed to determine the effects of different levels of Zn and Mn on yield, chlorophyll content, root growth and Zn and Mn concentration in different plant parts. Furthermore, the correlation between Zn and Mn in solution and plant was investigated.

MATERIALS AND METHODS

Experimental design: The nutrient culture method was used in this experiment. The experiment was performed at the Department of Land Management, Faculty of Agriculture, University Putra Malaysia (UPM). Sweet corn seeds hybrid 926 from Green World Genetics in Malaysia were used as the indicator plant. The seeds were soaked in water for 24 h and then geminated in rolled paper towels saturated with deionized water in the laboratory at 24°C. The paper towels were kept saturated for 5 days and the sweet corn seedlings were transplanted into 2 L capacity plastic pots containing nutrient solution at the rate of four seedlings per pot.

The basic nutrient solution was according to Trostle *et al.* (2001), which contained 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.1 mM KCL, 2 mM $Ca(NO_3)_2$, 0.0001 mM $CuSO_4$, 0.1 mM EDTAFe, 0.001 mM $MnSO_4$, 0.01 mM H_3BO_3 and 5×10^{-6} mM $(NH_4)_6Mo_7O_{24}$. pH was adjusted to 6.8 by using 0.1 M KOH or HCl solution. All combinations of Zn treatments (in the form of $ZnSO_4.7H_2O$) at levels of 0.0, 0.1, 1.0 and 10.0 mg L^{-1} and of Mn treatments (in the form of $MnSO_4.H_2O$) at levels of 0.0, 0.1, 1.0 and 10.0 mg L^{-1} were included. The nutrient solution was changed every three days. The experimental design was a randomized complete block consisting of 5 blocks (replications). The plants were grown at ambient sunlight. The temperature and humidity were 24-33°C and 70-88%, respectively.

Measurements: Plants were harvested 28 (V8- plants had 8 leaves) days after transplanting. The roots and shoots were then separated. The plant samples were rinsed with distilled water, dried at 70±2°C for 48 h, weighed and ashed at 300°C for 3 h followed by 500°C for 2 h in a muffle furnace. The ash was dissolved in concentrated HCl and 20% HNO_3. Zn and Mn were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima 8300, PerkinElmer, USA).

For chlorophyll content analysis, the fresh leaves (0.2 g) were put into the 20 mL glass vials. 5 mL concentrated dimethyl sulfoxide (DMSO) was added and the glass vials placed in an oven at 70°C for 1 h. Under the wavelength of 645 and 663 nm, the content of chlorophyll b and chlorophyll a were measured, respectively, using spectrophotometer (1000 series, Cecil CE 1011, Auckland, New Zealand). Arnon's equation Arnon (1949) was used to convert absorbance measurements to mg chlorophyll g^{-1} in leaf tissue.

Root parameters such as average root diameter, root length, root surface area and root volume were measured immediately after harvest by WinRHIZO 2012b software (Regent Instruments Inc., Quebec, Canada).

Calculations and statistical analysis: The dry weight of shoots and roots were multiplied by the concentrations of Zn and Mn to measure Zn and Mn uptake by shoots and roots, respectively.

Data were analyzed statistically by using SAS 9.2 software (SAS institute, Cary, NC, USA).

RESULTS

Mn concentration in roots and its translocation to upper plant parts showed a highly positive correlation with the Mn concentration in nutrient solution (Fig. 1).

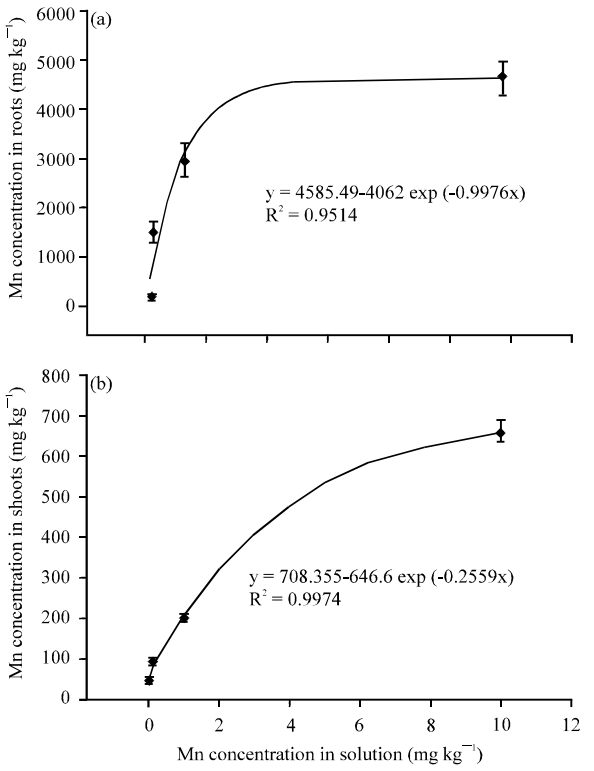

Fig. 1(a-b): Relationship between Mn supplies and Mn concentration in (a) Root and (b) Shoot of sweet corn palnts (vertical bars represent standard error)

Table 1: Dry weight (mg plant^{-1}) and total chlorophyll content (mg g^{-1} fresh weight) of sweet corn plants in nutrient solution with different Zn and Mn levels

Zn	Mn	Dry weight (mg plant^{-1})	Total chlorophyll content (mg g^{-1} fresh weight)
Treatments (mg L^{-1})			
0	0	136.0^{bc}	0.651^{ab}
	0.1	169.3^{abc}	0.514^{abc}
	1	221.0^{a}	0.528^{abc}
	10	152.7^{abc}	0.472^{abc}
0.1	0	126.0^{bc}	0.423^{abc}
	0.1	132.3^{bc}	0.394^{bc}
	1	167.7^{abc}	0.431^{abc}
	10	132.0^{bc}	0.683^{a}
1	0	200.7^{ab}	0.605^{ab}
	0.1	182.7^{abc}	0.638^{ab}
	1	182.3^{abc}	0.406^{bc}
	10	183.0^{abc}	0.457^{abc}
10	0	109.7^{c}	0.332^{c}
	0.1	158.3^{abc}	0.322^{c}
	1	134.7^{bc}	0.510^{abc}
	10	162.3^{abc}	0.372^{bc}

Same letter within each column indicates no significant difference between treatments ($p>0.05$)

Mn concentrations in roots and shoots were significantly different from each other at different levels of Mn in nutrient solution ($p\leq0.05$).

Zinc concentrations in roots and shoots were increased with increasing Zn concentration in nutrient solution with a very high non-linear correlation (Fig. 2). These correlations are higher than those observed during Mn uptake (Fig. 1). Zn concentrations in roots and upper plant parts were significantly different from each other at different levels of Zn in nutrient solution ($p\leq0.05$) except of Zn_0 and $Zn_{0.1}$ treatments in roots which did not show any significant difference ($p>0.05$). Zn and Mn concentrations reached a maximum in roots and shoots at highest Zn and Mn levels, respectively.

A high positive correlation was observed between Mn concentration in nutrient solution and Zn concentrations in roots and shoots of plants (Fig. 3). However, there were no significant differences in Zn concentrations of roots and shoots among those treatments receiving different levels of Mn. Zn concentration in roots and shoots followed the same trend but R^2 is higher in shoots as compared to roots. Zn concentration in roots was higher than shoots in all treatments.

Figure 4a revealed a strong negative correlation between Zn concentration in nutrient solution and Mn concentration in roots. The differences between all the treatments were significant ($p\leq0.05$) except of between $Zn_{0.1}$ and Zn_1 treatments. Mn concentration in roots was 3760 mg kg^{-1} in Zn_0 treatment and reached to 500 mg kg^{-1} in Zn_{10} treatment. The Mn uptake by roots in Zn_1 and Zn_{10} treatments were only 55 and 13% of the uptake in Zn_0 treatment.

Mn concentration in shoots was 265 mg kg^{-1} in Zn_0 treatment and decreased to 238 mg kg^{-1} with increasing

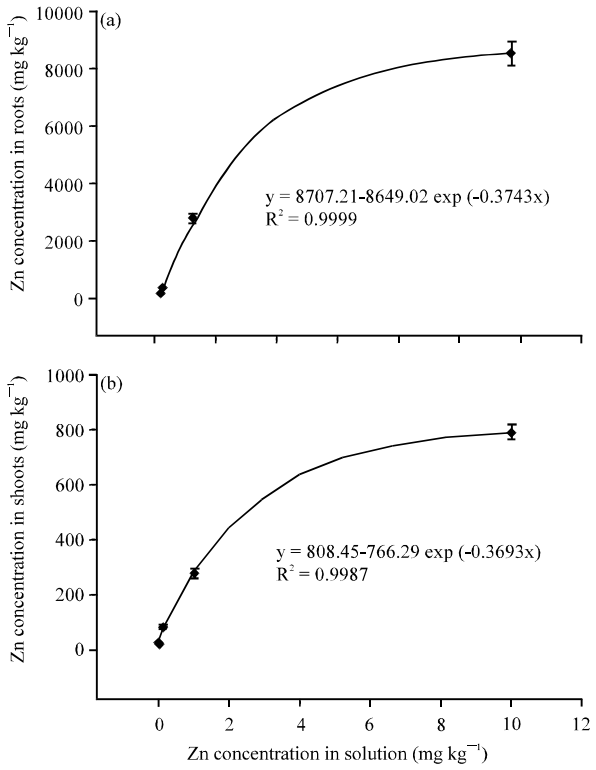

Fig. 2(a-b): Relationship between Zn supplies and Zn concentration in (a) Root and (b) Shoot of sweet corn palnts (vertical bars represent standard error)

Zn concentration in nutrient solution at the rate of 0.1 mg L^{-1}. The highest and the lowest concentration of Mn in shoots were observed in Zn_1 and Zn_{10} treatments, respectively. The differences of Mn concentrations in shoots between all the Zn treatments

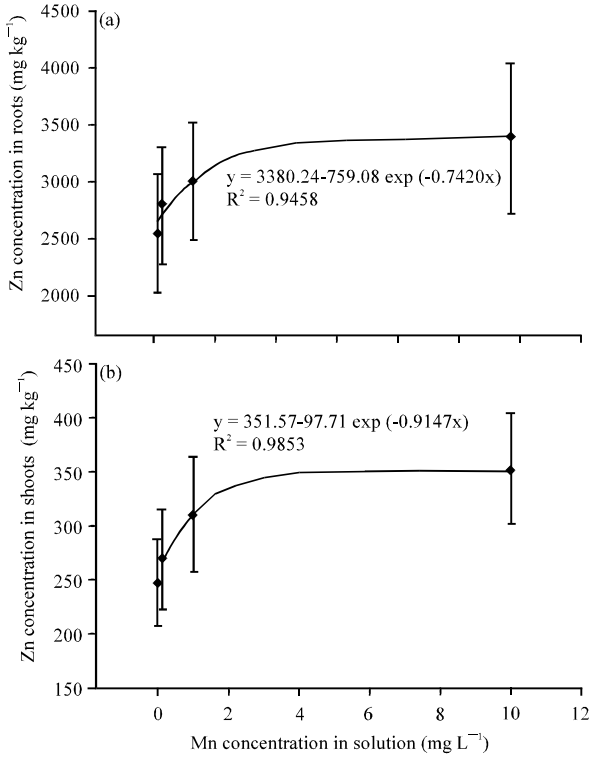

Fig. 3(a-b): Relationship between Mn supplies and Zn concentration in (a) Root and (b) Shoot of sweet corn palnts (vertical bars represent standard error)

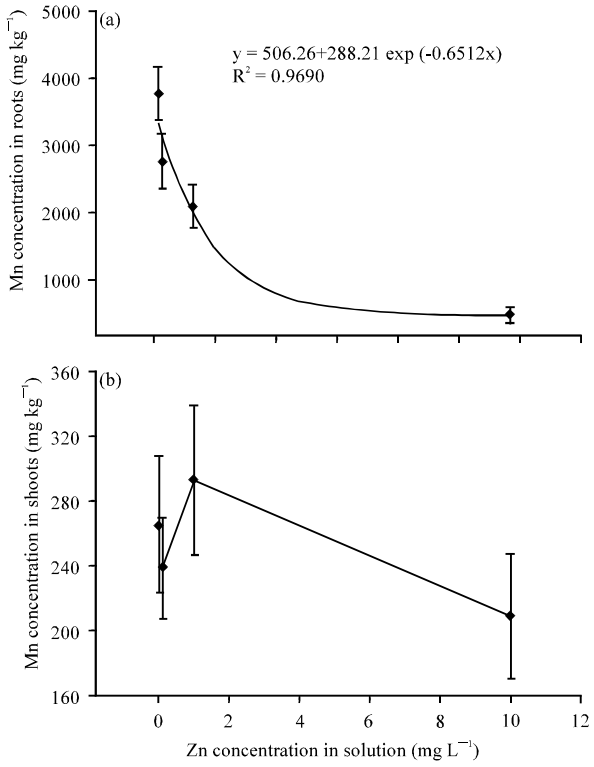

Fig. 4(a-b): Relationship between Zn supplies and Mn concentration in (a) Root and (b) Shoot of sweet corn palnts (vertical bars represent standard error)

were not significant ($p>0.05$) (Fig. 4b). Mn concentration in roots was higher than shoots in all treatments.

The highest dry matter yield was observed in Zn_0Mn_1 treatment (Table 1). Mn and Zn concentration in shoots of this treatment were 204.7 and 28.7 μg g^{-1}, respectively, which were within the normal range. Plants showed the lowest dry matter yield in $Zn_{10}Mn_0$ treatment (Table 1). This treatment had the lowest Mn concentration and Mn/Zn ratio in shoots which were 10.3 μg g^{-1} and 0.02, respectively (Table 2).

The lowest total chlorophyll content of 0.322 mg g^{-1} fresh weight was recorded under $Zn_{10}Mn_{0.1}$ treatment (Table 1).

The ratio of the uptake of Mn in roots and shoots showed a high negative correlation with Zn concentration in nutrient solution (Fig. 5a). The differences between all the treatments were significant ($p\leq0.05$). The lowest root/shoot Mn uptake ratio (0.14) was recorded in $Zn_{10}Mn_{0.1}$ treatment and this ratio reached a maximum (4.5) in Zn_0Mn_1 treatment (Table 2).

From R^2 in Fig. 5b, it is obvious that a close relationship exists between Zn concentration in solution and root/shoot Zn uptake ratio. This ratio increased with increasing Zn level but Zn_0 with $Zn_{0.1}$ and Zn_1 with Zn_{10} did not show a significant difference with each other ($p>0.05$). With enhancing the Zn concentration in solution, root/shoot Zn uptake ratio increased linearly until 1 mg L^{-1} level of Zn and then reached a plateau from 1-10 mg L^{-1} Zn level. The highest ratio of the uptake of Zn in roots and shoots (1.81) was observed in $Zn_{10}Mn_{10}$ treatment while the lowest one (0.4) was recorded in $Zn_{0.1}Mn_{0.1}$ treatment (Table 2). Mn concentration in solution did not have any effect on the aforementioned ratio (data not shown).

$Zn_{0.1}Mn_{10}$ treatment had the highest and $Zn_{10}Mn_0$ treatment had the lowest Mn concentration in roots which was 6433 and 25 μg g^{-1}, respectively (Table 2).

The lowest Zn concentration in roots was recorded in Zn_0Mn_0 treatment which was 58 μg g^{-1}, increasing thereafter with increasing Zn concentration in nutrient solution and reached maximum concentration of 10246 μg g^{-1} in $Zn_{10}Mn_{10}$ treatment (Table 2).

Table 2: Mn and Zn concentration (μg g^{-1}) in leaves and roots, Mn/Zn ratio in leaves, root/shoot Mn uptake ratio and root/shoot Zn uptake ratio of sweet corn plants in nutrient solution with different Zn and Mn levels

		Concentration in leaves (μg g^{-1})		Concentration in roots (μg g^{-1})			Root/Shoot uptake ratio	
Zn	Mn	Zn	Mn	Zn	Mn	Mn/Zn ratio in leaves	Zn	Mn
Treatments (mg L^{-1})								
0	0	47.7^{ef}	36.3^{fg}	119^{e}	58^{e}	1.94^{ef}	0.44^{def}	0.46^{e}
	0.1	94.0^{def}	20.3^{g}	3522^{bc}	88^{e}	5.61^{bc}	4.40^{a}	0.62^{cde}
	1	204.7^{de}	28.7^{fg}	5932^{a}	121^{e}	7.34^{b}	4.50^{a}	0.67^{bcde}
	10	716.0^{ab}	27.7^{fg}	5468^{a}	98^{e}	26.21^{a}	0.99^{def}	0.44^{e}
0.1	0	96.0^{def}	66.0^{fg}	52^{e}	339de	1.88^{ef}	0.17^{f}	0.57^{cde}
	0.1	120.0^{def}	93.7^{efg}	1164de	273de	1.35^{ef}	1.20^{cd}	0.40^{e}
	1	251.0^{d}	87.0^{efg}	3396^{bc}	431de	2.98^{de}	2.03^{bc}	0.74^{bcde}
	10	487.3^{c}	97.3^{efg}	6433^{a}	308de	4.69^{cd}	2.69^{b}	0.57^{de}
1	0	28.0^{f}	232.3^{de}	55^{e}	2460^{cde}	0.13^{f}	0.23^{ef}	1.44^{ab}
	0.1	133.0^{def}	176.7^{def}	1188de	2546^{cde}	0.76^{ef}	1.10^{cde}	1.77^{a}
	1	208.7^{d}	273.3^{cd}	2214^{cd}	3170^{c}	0.87^{ef}	1.35^{cd}	1.39^{abc}
	10	802.3^{a}	423.7^{c}	4895^{ab}	2882^{cd}	1.91^{ef}	1.16^{cde}	1.33^{abcd}
10	0	10.3^{f}	656.7^{b}	25^{e}	7259^{b}	0.02^{f}	0.19^{f}	1.12^{abcde}
	0.1	39.7^{f}	788.3^{ab}	42^{e}	8237^{ab}	0.05^{f}	0.14^{f}	1.37^{abc}
	1	157.0^{def}	854.0^{a}	253^{e}	8267^{ab}	0.19^{f}	0.24^{ef}	1.65^{a}
	10	628.0^{bc}	859.0^{a}	1681de	10246^{a}	0.74^{ef}	0.48^{def}	1.81^{a}

Same letter within each column indicates no significant difference between treatments ($p>0.05$)

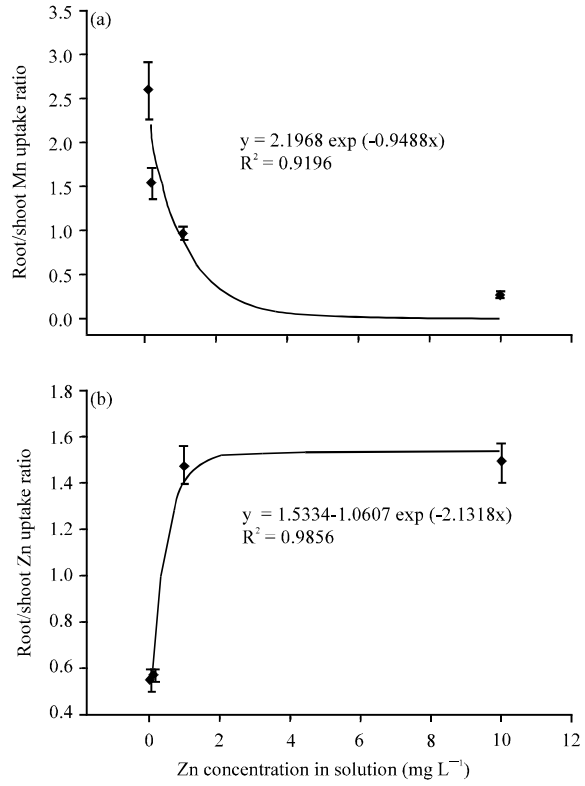

Fig. 5(a-b): Relationship between Zn supplies and root/shoot (a) Mn uptake ratio and (b) Zn uptake ratio of sweet corn palnts (vertical bars represent standard error)

DISCUSSION

The non-linear correlations between Mn concentration in tissues and Mn concentration in solution demonstrated that the Mn uptake for sweet corn at lower Mn concentration in the nutrient solution did not enhanced as the same rates as at higher Mn concentrations. It also showed that plants could uptake Mn passively and unrestrictedly in the whole levels of Mn concentrations in the nutrient solution. Although, this unrestricted uptake can lead to Mn toxicity at high levels of Mn in solution. Limited uptake of Mn by roots, regulated translocation of excess Mn from roots to upper plant parts or plant tissue tolerance to high Mn concentrations are the mechanisms in plants to tolerate to Mn toxicity (El-Jaoual and Cox, 1998). Similar results were obtained by Scherer and Hofner (1980) in maize, El-Fouly *et al.* (2001) in sunflower and Lombnaes and Singh (2003) in barely and oat.

Increased Zn concentration in roots and shoots with increasing Zn concentration in nutrient solution was also observed by Soltangheisi *et al.* (2013) in sweet corn and El-Fouly *et al.* (2001) in sunflower.

In this study, Zn concentrations in roots and shoots increased with increasing Mn concentration in nutrient solution but this positive relationship is controversial. Lombnaes and Singh (2003) demonstrated that Zn was enhanced in barely under Mn deficiency conditions. Quartin *et al.* (2001) in triticale and wheat observed that different levels of available Mn did not have a significant effect on whole plant Zn, while Zn concentration in roots was increased. Hawf and Schmid (1967) showed that different concentrations of Mn in solution had an effect on ^{65}Zn uptake of bush bean plants only at high concentration, but did not change the internal distribution. In contrast, De Varennes *et al.* (2001) reported a reduction in Zn concentration of annual medic with increasing Mn in nutrient solution. Barben *et al.* (2011) observed the same results in potato. Singh and

Steenberg (1974) demonstrated that the uptake of ^{65}Zn and total Zn of roots, sheaths and blades of maize were not affected by Mn application.

It seems that the effect of Zn on Mn concentration in shoots was less pronounced up to 1 mg L^{-1} Zn. Increasing Zn concentration in nutrient solution caused the reduction of Mn absorbed by corn and prevented Mn toxicity. Zn and Mn competed for the same absorption site and the decreased Mn uptake by roots with increasing Zn concentration in nutrient solution could be attributed to the ionic competition between Mn and Zn during the process of absorption. On the other hand, Zn appears to have little influence on the distribution and translocation of Mn in corn and as a result, there was not a correlation between Zn concentration in nutrient solution and Mn concentration in shoots. These results are comparable to those obtained by Yoshiaki and Ando (1968) in rice, Singh and Steenberg (1974) and Welch and Norvell (1993) in barely, Imtiaz *et al.* (2003) in wheat, Das *et al.* (2005) in *Stevia rebaudiana*, Adiloglu (2006) in maize and Barben *et al.* (2010a, b) in potato. Barben *et al.* (2011) in potato showed that root Mn decreased and shoot Mn increased as solution Zn concentration enhanced. Gunes *et al.* (1998) found that plant Mn of tomato was not influenced by enhancement of Zn in nutrient solution. Contrary results were reported by Lopez-Millan *et al.* (2005). They demonstrated that increasing Zn supply in nutrient solution from 0-5 μM caused the higher Mn concentration in roots and leaves of *Medicago truncatula*.

Zn_0Mn_1 treatment showed the highest dry matter yield. Mn and Zn concentrations in shoots of this treatment were within the normal range. Mn/Zn ratio in this treatment (7.34) was also within the sufficiency range. The normal range of Mn/Zn ratio is between 0.1-10 for obtaining 90% of the highest yield according to Yoshiaki and Ando (1968). The lowest dry matter yield in $Zn_{10}Mn_0$ treatment was due to Mn deficiency. In this treatment Mn concentrations and Mn/Zn ratio in shoots were below the normal range. The optimum range of Mn and Zn in whole tops of corn plants in this growth stage is between 20-300 and 20-60 μg g^{-1}, respectively (Jones *et al*., 1991). The reduction in yield in this treatment, was related to the metabolic function of Mn in the plant. Mn plays a pivotal role in photosynthesis, activity of enzymes, ribosome structure, carbohydrate metabolism, auxin catabolism and in fatty acid and carotenoid synthesis. In Zn_0Mn_{10} treatment the Mn/Zn ratio was higher than the critical range (26.21) and consequently, the yield was 69% of the highest yield.

The lowest total chlorophyll content was recorded under $Zn_{10}Mn_{0.1}$ treatment while it showed the optimum Mn and Zn concentration but a very low Mn/Zn ratio. This indicates that the ratio of Mn to Zn is a better indicator of Zn-Mn nutritional status than Zn or Mn concentration alone. The decreased chlorophyll content in these treatments was probably caused by iron and Zn interaction in the chlorophyll biosynthetic pathway and needs further investigations.

Investigation of the correlation between root/shoot Mn uptake ratio and Zn concentration in solution shows that Zn application adversely affects translocation of Mn from root to shoot. Zn can restrict the translocation of excess Mn to shoots and reduce Mn toxicity in plant tissue.

Mn concentration in roots increased with increasing Mn concentration in nutrient solution. Same result was observed by Lombnaes and Singh (2003) in barely and oat. The lowest Mn concentrations in Zn_{10} treatment in different levels of Mn application showed that the total uptake of Mn from substrate to roots was decreased by the presence of Zn. Accordingly, Mn uptake by roots depends not only on Mn level in nutrient solution but also on the balance of Mn and Zn in the growth medium. Yoshiaki and Ando (1968) in rice and Barben *et al.* (2010a, b) in potato found that Mn uptake by plants significantly reduced with the enhancement of Zn in nutrient solution.

Different levels of Zn and Mn in nutrient solution did not have any significant effect on root parameters such as average root diameter, root length, root surface area and root volume (data not shown). El-Fouly *et al.* (2001) demonstrated that Zn did not show a significant effect on stem and root length and root size, while Mn application increased stem length, root length and root size.

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