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The Response of Pea Plant (*Pisum sativum*) to Manganese Toxicity in Solution Culture

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Abstract: In this research the effects of 0, 25, 50, 100, 200 and 300 ppm of manganese concentration on *Pisum sativum*. L. (c.v.qazvin) plants growth, in nutrient solution (PH 5) on the controlled condition during 15 days have been studied. Maximum yields (dry weight of shoot and root) were obtained at 25 ppm manganese supplied in nutrient solution in comparasion with controls. The shoot/root weight ratio (SRW) showed decrease with increasing manganese in nutrient solution, this decrease in (SRW) confirmed that in toxic levels of manganese shoot have been more affected than roots of pea plant. Length of shoots and roots were retarded below and above 25 ppm of manganese supplied in nutrient solution. Manganese content in young and old leaves and roots of pea plants were determined. The distribution pattern of manganese in 5 treatments was different. Old leaves had the highest, while the young leaves had the lowest manganese content. In all treatments manganese uptake has been increased proportionally with manganese concentration in nutrient solution. Excess manganese above 50 ppm in nutrient solution has caused to unfavorable symptoms including chlorotic (yellowed) areas, brown and necrotic spots and decrease in plant size.

Key words: Manganese, tsoxicity symptoms, *Pisum sativum*, pea plant

INTRODUCTION

Manganese is an essentioal micronutrient for plants, but when is excess, it has some toxic effects on plants growth. Manganese toxicity is one of the important factors limiting plant growth on acidic soils of pH<5.5 (Foy *et al*., 1978). For a wide range of plant species, formation of brown spots is part of a characteristic development of Mn toxicity symptoms in older leaves. The subsequent development of chlorosis and necrosis and finally leaf shedding occurs before a reduction in vegetative growth on the whole plant level (Horst, 1988). Analysis of the Mn-induced formation of brown spots revealed the presence of oxidized Mn and oxidized phenols, especially in the cell wall of the epidermis layer (Horiguchi, 1987; Wissemeier and Horest, 1992). Human practices have raised Mn content and availability in many soils. Long-term and heavy dose applications of sewage-sludge (biosoils) or other organic amendments to agricultural soils and soil anaerobic condition such as water logging or poor drainage may also lead to an increase in the content or availability of Mn and other heavy metals (Ramachandran and D'Souza, 1997). Manganese availability to plants is dependent on several factors including soil pH, redox potential, soil moisture and microbiological activity in the soil (Wang *et al*., 2001). Manganese uptake by plants mainly occurs in the reduced-bivalent form, thus its availability increase in acidic soils or anaerobic conditions. High Mn levels in soil may lead to plant nutrient imbalances, especially in relation to other divalent cations such as Mg^{+2} and Ca^{+2} (Marschner, 1995; Cenni *et al*., 1998) and Zn (de Varennes *et al*., 2001). In general, nutrient solution uptake, especially in relation to elements entering the roots by diffusion, may be hampered by Mn due to Mn inhibition of root hair production and reduction of stomata dimensions (Lidon, 2002). Plants may differ considerably within and between species in Mn tolerance due to both genetic characteristics and environmental factors such as nutrient availability in the soils (Goss and Carvalho, 1992). (Mahmoud and Grime, 1977) observed that the susceptibility of a given species to high Mn levels was related to their ecology and the ability of the species to tolerate acidic soils.

Our objective of this study were to determine ideal levels of manganese concentration for growth of pea plant, to maintain toxic levels of Mn and to show the Mn toxicity symptoms in pea plants were grown in nutrient solution.

MATERIALS AND METHODS

To do the experiment seeds of pea plants were surface strelized with 98% H_2SO_4 for 15 min, rainesed carefully with tap water and ancubated at 25°C to germinate for 3 days. After germination, the seedlings

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were transfered to plastic pots containing 200 mL of nutrient solution (4 seeds in each pot and each treatment with 3 replicates) in the controlled condition (period of day/night, 12/12 h, at 25°C, a relative humidity between 50-60% and light density was 20 μ mol m^{-2} s^{-1}). Seedlings were irrigated 5 times per week with the nutrient solution (pH 5) for the period 15 days; then the plants were harvested and the visible symptoms of Mn toxicity, the length, dry weight of shoots and roots, Shoot/Root Dry Weigh ratio (SRW) and Mn content of different parts of plants were determined. Tissues was dried at 70°C, weighed, ashed at 500°C for 12 h, dissolved in 0.1N HCl and analyzed for Mn content by atomic absorbtion spectrophotometry.

RESULTS AND DISCUSSION

Mn toxicity symptoms in pea plants are shown as brown and necrotic spots on older leaves surrounded by chlorotic zones. In higher Mn concentrations (200 and 300 ppm), the brown spots have turn to dark-brown and necrotic spots, leaf margins were withered and lastly the leaf were dried and fell (Table 1).

Table 1, has indicated that in 25 ppm Mn in nutrient solution pea plants has not shown any visible symptom; in 50 ppm also has not appeared visible symptoms but the growth has reduced. In concentrations of 100 ppm and above the toxicity symptoms were severe with increasing the Mn concentration in nutrient solution. Also, the growth were reduced, whereby in 300 ppm of Mn concentration in nutrient solution pea plants were died. In pea plant, Mn toxicity symptoms at the first have appeared in old leaves and symptoms were severe in old leaves than young leaves, because Mn is an immobile element. Therefore, Mn, after transfered to shoots and old leaves from the roots, can not be retransferred to young leaves, thus Mn has accumulated in old leaves and has induced Mn toxicity in these leaves (old leaves). The Mn toxicity symptoms observed in 100 and 200 ppm of treatments, were similar to those previously has been described for rape seed (Bjarnason *et al*., 1972). Although, Mn toxicity symptoms varies considerablly among plant species, but some of the symptoms such as chlorosis and brown spots were similar to those that has been reported for wheat and soybean (Heenan and Campbell, 1981). Mn uptake: The results has showed that Mn uptake has increased with increasing Mn concentration in nutrient solutions (Fig. 1). Manganese has taken up readily by the roots and has translocated to the leaves. Accumulation of Mn in the leaves appear to be cumulative in pea plant where Mn concentration has increased with leaf age since, Mn readily transported from root to shoot but has not remobilized from old leaves to young leaves.

Table 1: Mn Toxicity symptoms and level of toxicity in Pea plants were grown in nutrient solution containing different concentratsions of Mn for 15 days

Treatments (ppm)	Mn toxicity symptoms	Level of toxicity
0	None	-
25	None	-
50	None	Effective concentration (E.C)
100	Chlorotic areas in old leaves	Toxicity concentration (T.C)
200	Brown and necrotic spots, chlorotic areas, leaf margins withered	Toxicity concentration (T.C)
300	The plant turn to dark, dry and shadded	Lethal concentration (L.C)

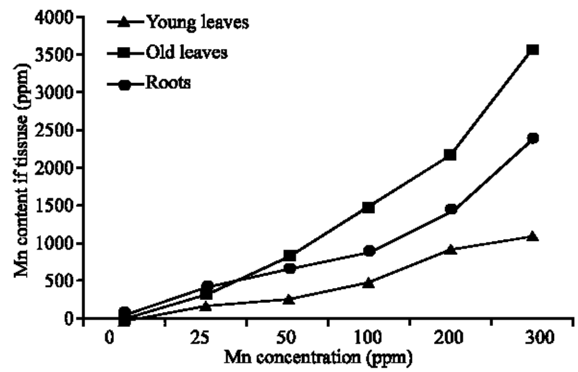

Fig. 1: Manganese content, in roots, young and old leaves of pea plants has grown in solutions containing 0, 25, 50, 100, 200 and 300 ppm of Mn for the period of 15 days. The data indicates the mean ±SE

Mn content has been increased in old leaves, roots and young leaves with increasing Mn concentration in culture solution, respectively.

These results are agree with those that Clark (1977) and Furlani and Clark (1981) has shown in sorghums has grown on excess Mn. Romney and Toth (1954) have also found that more Mn was present in mature leaves than in young leaves of buckwheat, soybean and sunflower plants and the stem contained less Mn. These results suggest that Mn is less mobile in the sive tubes (Vlamis and Williams, 1964). A possible explantation for this variation may be the differential behaviouar of plants genotypes in the translocation of Mn within plants (Singh and Steenberg, 1997). Therefore, occurrence of Mn toxicity dose not depend only on the amount of available Mn and the rate Mn uptake, but also depends on susceptibility and tolerance of plant to Mn content in tissues. So, according to many reports (Lohnis, 1960) toxicity symptoms of Mn in different plants occurs in different levels of tissues Mn contents that differs from 400-2000 ppm (Singh and Steenberg, 1997). In our

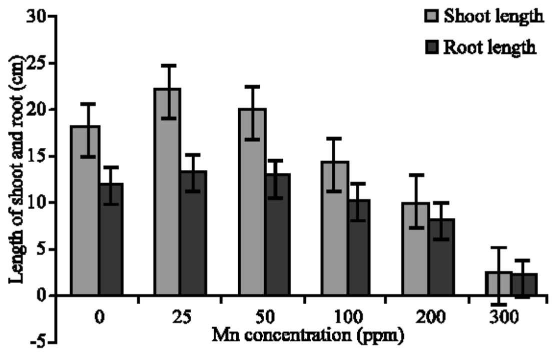

Fig. 2: Effect of different Mn concentrations on the changes of the length of shoots and roots of *Pisum sativum* L. during 15 days. The data represents the mean of 3 replicates ±SE

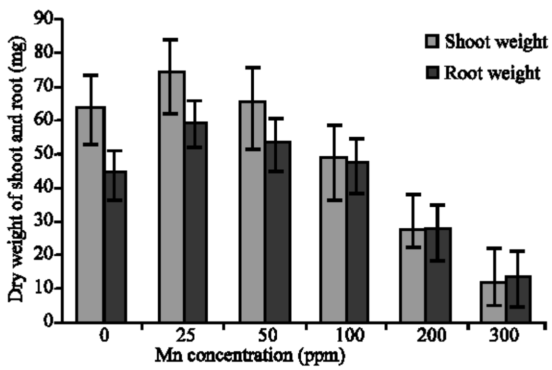

Fig. 3: Effects of different Mn concentrations on the dry weight of shoots and roots of *Pisum sativum* L. during 15 days. Data represents the mean of 3 replicates ± SE

experiment pea plants did not show toxicity symptoms unless the level of Mn in leaves has reched to 1500 ppm, therefore, the pea plant has a good tolerance to excess Mn. One of the important factor that influence Mn uptake by plants is competition between Mg^{2+} and other bivalent cations.

The changes of the length and dry weight of shoots and roots: The results has showed that the length and dry weight of the roots and shoots were the highest in 25 ppm of Mn concentration in nutrient solution and in concentrations above 25 ppm both the length and dry weight have decreased with increasing Mn concentration in nutrient solution whereby the lowest length and dry weight has observed in 300 ppm (Fig. 2 and 3).

The changes of dry weight of shoots and roots and the Shoot/Root Dry Weight ratio (SRW) have been proved to be the suitable parameters for determination of

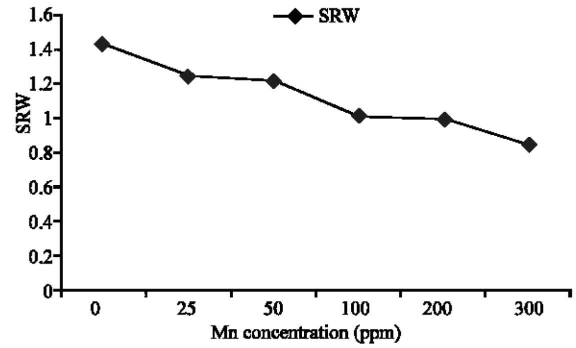

Fig. 4: Shoot/Root Dry Weigh ratio (SRW) of *Pisum sativum* L. exposed to different concentrations of Mn during 15 days

plant resistance to Mn toxicity. These results showed that SRW has decreased with increasing of Mn concentration in nutrient solution (Fig. 4).

This decrease in SRW confirmed that in toxic level of Mn the shoot were more affected than roots. Also (Mgema and Clark, 1993) showed that Mn concentration in sorghum shoots was higher than in roots when plant were grown with excess Mn Therefore, shoot were more affected than roots. This results in pea and sorghum contrast with those result of soybean (Kohno *et al*., 1984) and wheat (Macfie and Taylor, 1989), where roots contained higher Mn concentration than shoot and Therefore, root were more affected than shoot. This difference may be due to absorption and accumulation Mn on external roots surfaces plants, in this latter studies Therefore, accumulation Mn in their roots induced high toxicity in roots than shoots.

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