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Investigation on Zinc, Iron and Manganese Interactions by [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] Ratios in Soybean Roots and Shoots

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ABSTRACT

Micronutrient ratios indicate the presence of known and unknown relationships between the elements. Therefore, an investigation was taken up to study uptake limitation of zinc, iron and manganese by [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ratios in roots and shoots of soybean in a pot experiment Kermanshah, Iran. The experimental design was a 3×3×3 factorial experiment with 81 pots laid out in randomized complete block with three replications. Treatments included three rates of zinc, iron and manganese. After eight weeks, plants were harvested at 1 cm above the soil surface and samples were used to measure the micronutrients and then, [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ratios were calculated. The results showed that zinc application up to 4 mg kg^{-1} soil had no effect on [Zn]/[Fe] ratio, but increases [Zn]/[Mn] ratio in soybean roots. Also, with Fe application up to 8 mg kg^{-1} soil, [Zn]/[Mn] ratio was reduced. Manganese fertilization up to 15 mg kg^{-1} soil tended slight increase in [Zn]/[Fe] ratio and excess amount had no effect on this ratio. In addition, [Zn]/[Fe] and [Zn]/[Mn] ratios linearly increased with zinc application. Zinc applied decreased, [Fe]/[Mn] ratios in soybean shoots. On the other hand, use of 4 mg Fe kg^{-1} soil reduced [Zn]/[Fe] ratio and increased [Fe]/[Mn] and [Zn]/[Mn] ratios in soybean shoots. Application of 30 mg Mn kg^{-1} soil, increased [Zn]/[Fe] ratio. Increase in [Zn]/[Fe] and [Zn]/[Mn] ratios enhanced shoot dry weight in soybean plants. The highest of shoot dry weight of soybean was obtained in [Fe]/[Mn] ratio range of 1.0 to 1.5.

Key words: Antagonistic effects, elements ratio, nutrient balance, shoots dry weight, soybean

INTRODUCTION

Micronutrient concentration in plant organs is related to plant growth stage, micronutrient mobility and availability of micronutrient in soil. Antagonistic relationships between micronutrients such as iron with manganese (Ghasemi-Fasaei *et al*., 2005; Ronaghi and Ghasemi-Fasaei, 2007), zinc with iron (Kaya and Higgs, 2002; Deckers and Steinnes, 2004; Bernal *et al*., 2007) and zinc with manganese (Ming and Yin, 1992; Alloway, 2004) have been emphasized in previous studies. These antagonistic effects are the preventer for micronutrients uptake by plant roots and transfer to the shoots. Souza *et al*. (2005) reported that zinc and iron interaction leads to inhibition of absorption, transfer to leaves and utilization of iron and finally decreased synthesis of chlorophyll in the leaves. Micronutrient concentration in the soil not only increases the same micronutrient

uptake but also affects the absorption of other micro and macro nutrients in the soil (Graham and Webb, 1991). Therefore, fertilizers application must be done in ways that increases the balance of nutrients in the soil and plants (Khan and Zende, 1977; Carsky and Reid, 1990; Tandon, 1995). Nutrient concentration ratios were known as main diagnostic tool for determining the nutritional balance of plants (Beaufils, 1973). In the study of Ming and Yin (1992), this method was used for evaluation of nutrient balances and introducing disorder factors of nutrient balances in wheat plants. The purpose of this study was to determine the effects of zinc, iron and manganese application on [Zn], [Fe] and [Mn] ratios in soybean roots and shoots.

MATERIALS AND METHODS

The pot experiment was carried out in 2010 at the research field of the Islamic Azad University of Kermanshah province, Iran (34°23' N, 47°8' E; 1351 m elevation). The experimental design was a 3×3×3 factorial experiment based on Randomized Complete Block Design (RCBD) with three replicates in 81 pots. Surface soil was collected from an agricultural field and passed through a 2 mm mesh screen. The texture of the soil based on silty clay with pH 7.6, total organic matter 1.8%, Electrical Conductivity (Ece) 0.46 dsm^{-1}, total nitrogen 0.09%, available phosphorus 7.4 mg kg^{-1}, available potassium 435 mg kg^{-1}, zinc, iron and manganese 0.56, 5.1 and 3.2 mg kg^{-1}, respectively. The experiment consist of 27 treatments included of three rates of zinc (0, 4 and 8 mg Zn kg^{-1} as $ZnSO_4.7H_2O$), three rates of iron (0, 4 and 8 mg Fe kg^{-1} as $FeSO_4$) and three rates of manganese (0, 15 and 30 mg Mn kg^{-1} as $MnSO_4.4H_2O$). All pots were fertilized with 20 mg N kg^{-1} as NH_4NO_3, 40 mg P kg^{-1} as Ca $(H_2PO_4).2H_2O$. Six seeds of soybean (cv. Williams) inoculated with *Bradyrhizobium japonicum* and were sown directly in plastic pots containing 4 kg of the soil. After 48 days, plants were lifted and samples were washed in deionized water, then shoot and root were separated. For measure of dry weight samples was dried at 70°C and 48 h, weighed and incinerated at 550°C. For micronutrients determination, dry ash samples soluble in concentrated HNO_3 and $HClO_4$. The Zn, Fe and Mn contents were determined by Atomic Absorption Spectrometry (AAS) according to Kacar (1984). Then ratios of [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] in soybean roots and shoots were calculated. Excel software was used to draw figures.

Statistical analysis: Data for evaluated traits were statistically analyzed using a standard analysis of variance technique for factorial experiment based on randomized complete block design using the statistical software MSTATC. Means were separated by the Least Significance Difference (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Change in the ratios of [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] in soybean roots: The effect of zinc application on Zn, Fe and Mn ratios in soybean roots were shown in Fig. 1. Compared check treatment (0 mg Zn kg^{-1} soil) zinc application up to 8 mg Zn kg^{-1} soil increased [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ratios by 14.29, 26.28 and 10.23% respectively, while, use of 4 mg of zinc per kg soil had no effect on [Zn]/[Fe] ratio in soybean roots (Fig. 1a). Seemingly, the use of high doses of zinc can inhibit the absorption of iron by plant roots. Linear increase in [Zn]/[Mn] ratio indicates that with the use of zinc, Zn concentration in roots increased and at the same time, Fe concentration was reduced (Fig. 1b). A similar result was observed in [Fe]/[Mn] ratio (Fig. 1c). Korzeniowska and Stanislawska-Glubiak (2004) stated that the ratio of iron to manganese in acid soils usually would

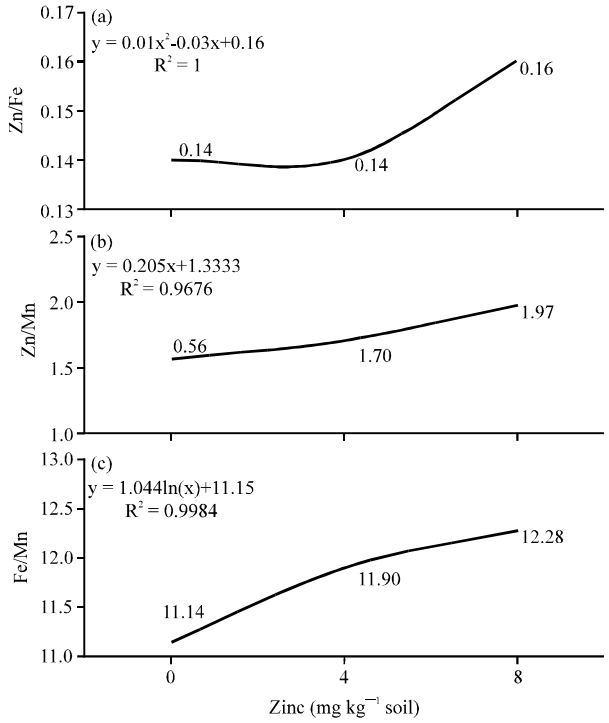

Fig. 1(a-c): Effects of zinc application on (a) [Zn]/[Fe], (b) [Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean roots

be less than 1.0 that this indicates the antagonistic effects between these two elements and iron deficiency in this soil type. In their experiment the ratio of iron and manganese were ranged 3-15. In addition, Kovacevic *et al.* (2004) revealed that [Fe]/[Mn] ratio varies in a range of 2.40-5.79. The [Zn]/[Fe] and [Zn]/Mn] ratios in soybean roots decreased when iron was applied. Indeed, these results show that zinc uptake by roots is limited by iron fertilization (Fig. 2a, b). Iron application up to 4 mg Fe kg^{-1} soil increased Fe concentration in roots and thereby increased the [Fe]/[Mn] ratio (Fig. 2c). But, with excess amount of iron (8 mg Fe kg^{-1} soil), [Fe]/[Mn] ratio decreased (14.22-11.91). Daneshnia *et al.* (1991) emphasized that excessive iron intake, particularly in calcareous soils, reduced uptake and transfer of micronutrients such as zinc, manganese and copper in pant roots. The effects of manganese application on [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ratios in soybean roots were shown in Fig. 3. Based on results obtained, [Zn]/[Fe] ratio in soybean roots increased with application of 15 mg Mn kg^{-1} soil and excess amount of manganese had no effect on this ratio (Fig. 3a). In contrast, compared control treatment manganese fertilization caused a sharp decline in [Zn]/[Mn] and [Fe]/[Mn] ratios in soybean roots by 147.83 and 177.49%, respectively (Fig. 3b, c). With the increase in manganese application, Mn concentration in soybean roots increased and in the other side, Zn and Fe concentrations in roots decreased, similarly.

Change in the ratios of [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] in soybean shoots: The impact of zinc fertilization on [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ratios in soybean shoots were shown in Fig. 4. In soybean shoots, [Zn]/[Fe] and [Zn]/[Mn] ratios increased 159.46 and 78.00% when zinc were used (Fig. 4a, b). Ali *et al.* (1998) and Wang and Jin (2007) reported that with zinc sulfate

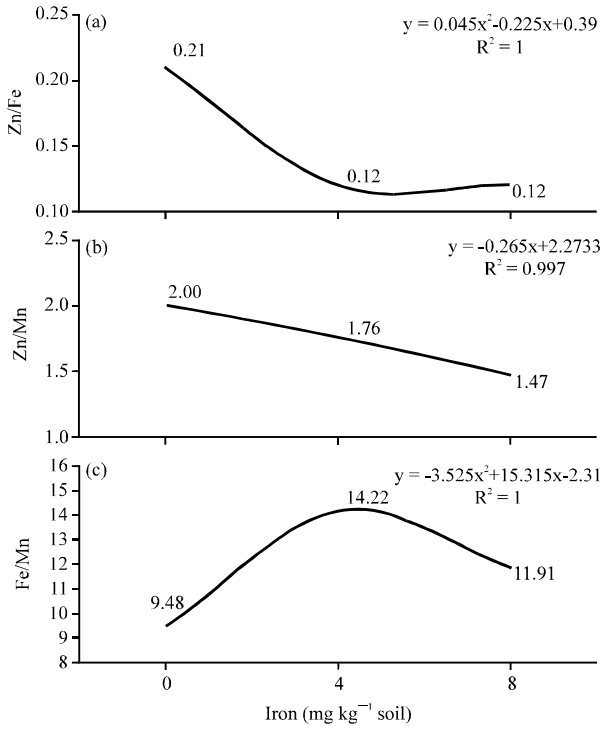

Fig. 2(a-c): Effects of iron application on (a) [Zn]/[Fe], (b) [Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean roots

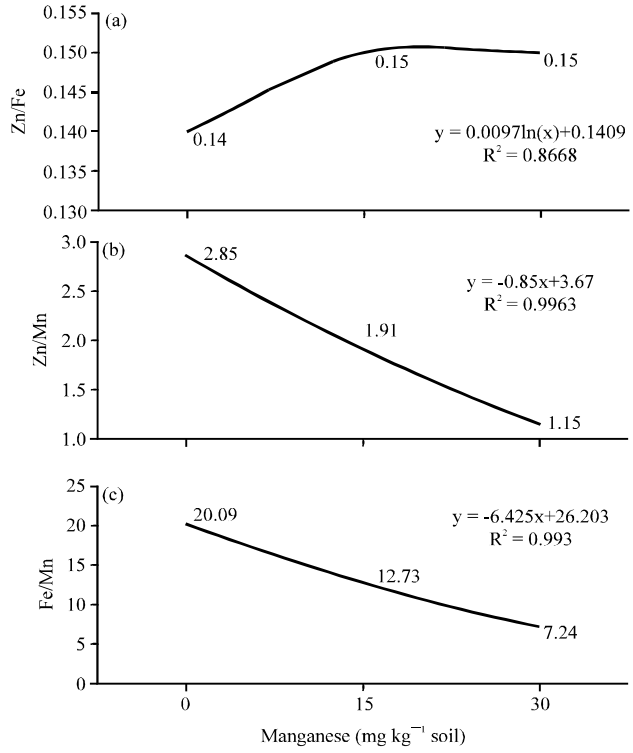

Fig. 3(a-c): Effects of manganese application on (a) [Zn]/[Fe], (b) [Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean roots

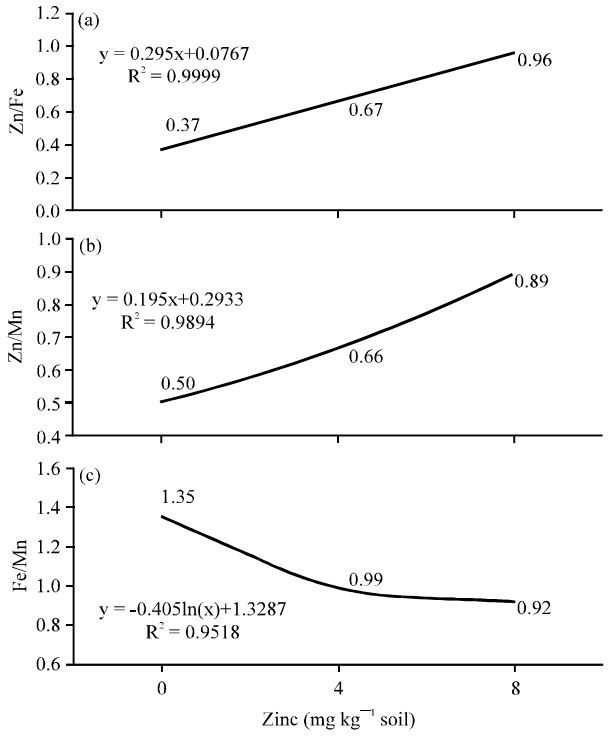

Fig. 4(a-c): Effects of zinc application on (a) [Zn]/[Fe], (b) [Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean shoots

application, zinc concentration in the plant shoots increased. Linear trends are the result of the impact of the zinc application on the concentration of the element in the soybean shoots. While the ratio of [Fe]/[Mn] in soybean shoots reduced at this condition (Fig. 4c). Kaya *et al.* (2000) reported that there are known and unknown antagonistic effects between zinc and other trace elements that prevent their absorption by the plant roots. In the iron fertilization study (Fig. 5), Fe concentration in soybean shoots increased in 4 mg Fe kg^{-1} soil and in excess amounts, concentration of this micronutrient was decreased, thereby [Zn]/[Fe] ratio reduced and [Fe]/[Mn] ratio enhanced (Fig. 5a, c). Ghasemi-Fasaei *et al.* (2002) reported that [Fe]/[Mn] ratio less than 0.4 in soybean shoots is index for imbalance and considered as a genotype tolerant to Fe chlorosis. Iron application improved the absorption of zinc and manganese uptake was limited, therefore, the result was that the reduced in [Zn]/[Mn] ratio in soybean shoots (Fig. 5b). Voogt and Sonneveld (2009) stated that with iron fertilization, manganese concentration in plant shoots is reduced. As similar results, manganese application reduced [Zn]/[Mn] and [Fe]/[Mn] ratios in soybean shoots by 77.84 and 78.86%, respectively (Fig. 6b, c), while, the response of [Zn]/[Fe] ratio to manganese fertilization was different (Fig. 6a). Furthermore, [Zn]/[Fe] ratio in shoots increased with 15 mg Mn kg^{-1} soil treatment and in excess amounts (up to 30 mg Mn kg^{-1} soil), this ratio was increased. In manganese treatment, the [Zn]/[Fe] ratio in soybean shoots was varied between 0.62-0.65. The effects of zinc, iron and manganese fertilization on plant dry weight of soybean were shown in Fig. 7. According to results obtained, the maximum total dry weight of soybean plants were recorded with application of 8 mg Zn and Fe kg^{-1} soil (Fig. 7a, b) while, the highest plant dry weight of soybean was achieved with 15 mg Mn kg^{-1} soil and in excess amounts of manganese, total dry weight was decreased (Fig. 7c).

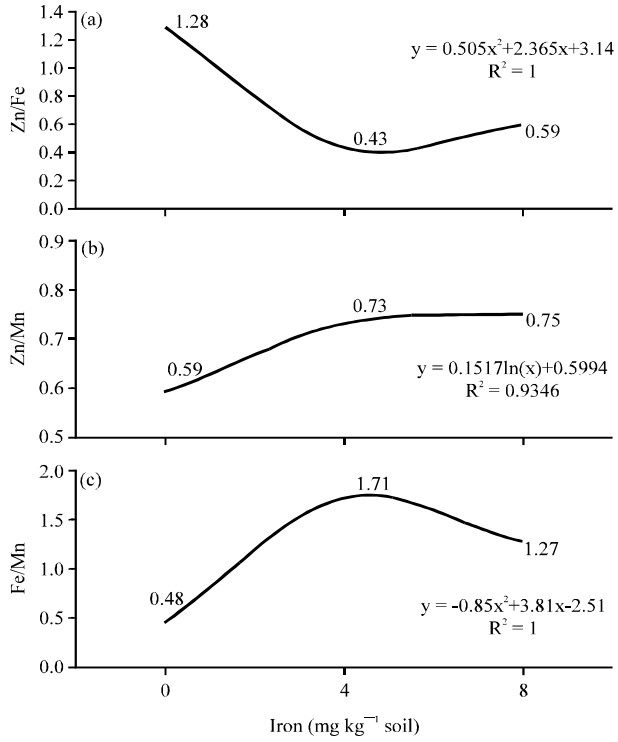

Fig. 5(a-c): Effects of iron application on (a) [Zn]/[Fe], (b)[Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean shoots

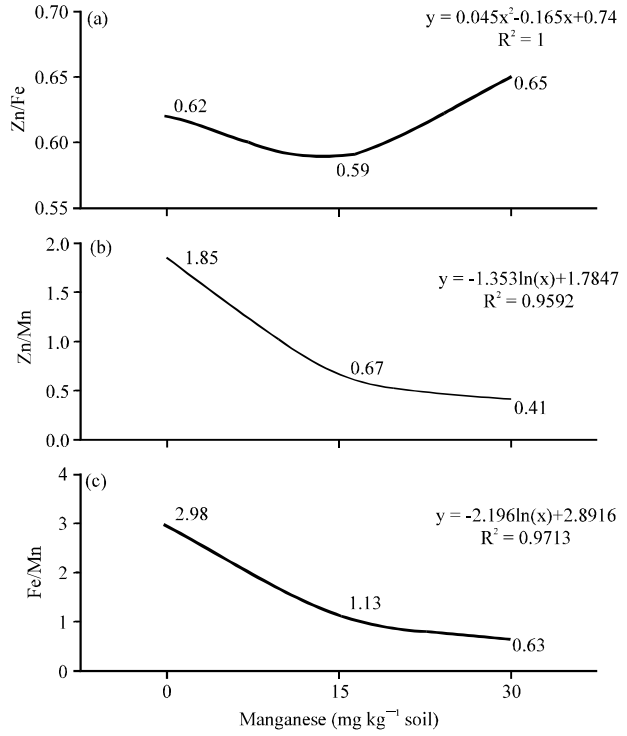

Fig. 6(a-c): Effects of manganese application on (a) [Zn]/[Fe], (b) [Zn]/[Mn] and (c) [Fe]/[Mn] ratios in soybean shoots

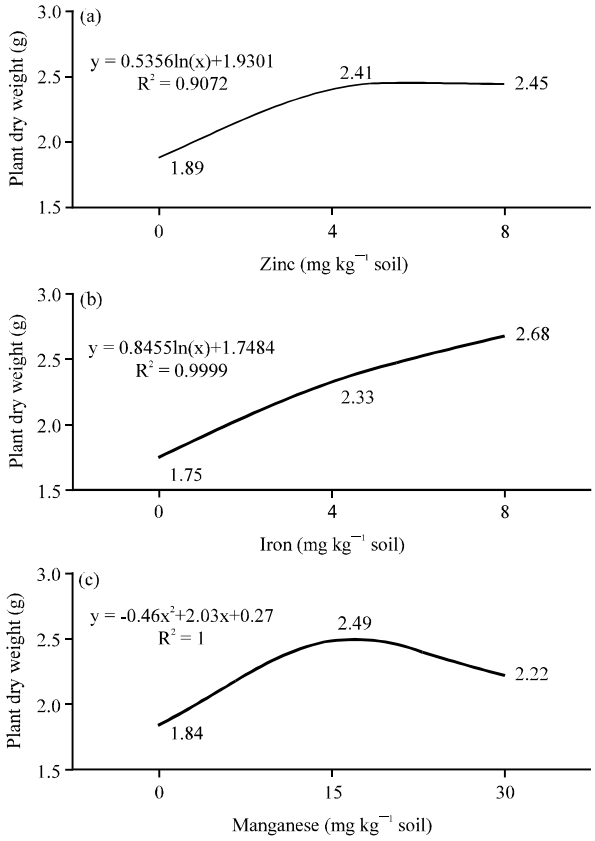

Fig. 7(a-c): Effects of (a) Zinc, (b) iron and (c) Manganese applications on total dry weight of soybean plants

CONCLUSION

In our experiment, in soybean roots the ratios of [Zn]/[Fe], [Zn]/[Mn] and [Fe]/[Mn] ranged 0.12-0.16, 1.15-2.85 and 7.24-20.09 and in soybean shoots ranged 037-1.28, 0.41-1.85 and 0.48-2.98, respectively. Indeed, in shoots ratio of [Zn]/[Fe] increased [Fe]/[Mn] and [Zn]/[Mn] decreased, this could be due to more transfer of manganese and/or poor ability transport of zinc and iron from the roots to shoots.

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