

INFLUENCE OF WATER DEFICIT ON IRON AND ZINC UPTAKE BY *Matricaria chamomilla* L.

Alireza Pirzad^{1*}, Reza Darvishzadeh¹, Iraj Bernousi¹, Abbas Hassani², and Nuray Sivritepe³

Iron and zinc deficiency are a yield-limiting factor with major implications for field crop production in many agricultural regions of the world; this production is essential source of Fe and Zn in human and animal diets. Two experiments were conducted under greenhouse conditions to evaluate Fe and Zn uptake by German chamomile (*Matricaria chamomilla* L.) grown under several drought conditions. Treatments were arranged in four irrigation regimes (95%, 80%, 65%, and 50% field capacity) and six irrigation disruptions (stem formation, flower onset, full bloom, first harvest, second harvest, and control without disruption) based on a randomized complete block design with five and four replicates in experiments 1 and 2, respectively. In Experiment 1, the irrigation regimes had no significant effect on Fe and Zn uptake by German chamomile, which reached total means of Fe (39.68 mg kg⁻¹) and Zn (29.15 mg kg⁻¹). In Experiment 2, the highest amount of Fe (39.0 mg kg⁻¹) was obtained from irrigation disruption at first harvest; this is similar to the results of irrigation disruption at flower onset and full bloom. However, irrigation disruption in stem formation (21.3 mg kg⁻¹), second harvest (12.95 mg kg⁻¹), and the control (7.79 mg kg⁻¹) had a decreasing trend of Fe uptake. The maximum value of Zn (40.0 mg kg⁻¹) occurred at irrigation disruption at the full bloom stage, which was also similar to irrigation disruption at flower onset and stem formation. However, later irrigation disruptions at the first and second harvest stages caused lower amounts of Zn uptake by chamomile. Therefore, the lowest Zn values were obtained from irrigation disruption at second harvest (5.0 mg kg⁻¹) and the control (5.5 mg kg⁻¹). Regressions between both Fe or Zn uptake and irrigation showed a binomial function.

Key words: Irrigation, Fe, *Matricaria chamomilla*, micronutrients, Zn.

Heavy metals are considered as one of the main sources of environmental pollution. In biological and environmental studies, metals are classified in two categories: essential metals, such as Fe and Zn; and nonessential or toxic metals, such as Pb and Cd. Essential metals can also produce toxic effects when metal intake concentrations is high (Angelova *et al.*, 2004). Iron is one of the most important micronutrients in the human diet and functions as a component of a number of proteins, including enzymes and hemoglobin (Institute of Medicine, Food and Nutrition Board, 2001). Plants are essential sources of Fe in human and animal diets and the Fe concentration in plants is often not enough to meet the daily dietary recommendations. Iron deficiency is a yield-limiting factor with major implications for field crop production in many agricultural regions of the world (Barton and Abadía, 2006). Krouma *et al.* (2003)

found differences in Fe reduction among five cultivars of common bean related to hydroponically induced Fe chlorosis, but no field chlorosis relationships were established in their study. An excessive Fe concentration causes vomiting, diarrhea, and also damages the intestine (Haider *et al.*, 2004). Zinc is one of the most important trace elements in the body for many biological functions; it is required as a catalytic component for more than 200 enzymes and as a structural constituent of many proteins, hormones, neuropeptides, hormone receptors, and probably polynucleotides (Fabris and Mocchegiani, 1995). However, Zn is one of the essential micronutrients required for plant growth. It is an integral component or activator of a number of enzymes that represent almost all plant enzymatic groups. High soil Zn concentrations, a frequent consequence of anthropogenic activities, adversely affect plant life processes and can result in decreased biomass production and deteriorated quality of production (Vysloužilová *et al.*, 2003).

Herbal or medicinal plant products in various forms have been available for hundreds of years to treat diseases in both Eastern and Western cultures. Plants are a rich source of secondary metabolites with medicinal and aromatic properties (Gómez-Galera *et al.*, 2007). The world market for herbal medicines, including herbal products and raw materials, is currently growing at an annual rate of 5% to 15%. This indicates a possible

¹Urmia University, Faculty of Agriculture, Department of Agronomy and Plant Breeding, Urmia 5715944931, Iran.

*Corresponding author (a.pirzad@urmia.ac.ir; alirezapirzad@yahoo.com).

²Urmia University, Faculty of Agriculture, Department of Horticulture, Urmia, Iran.

³Uludag University, Faculty of Agriculture, Department of Horticulture, Bursa 16059, Turkey.

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growing demand for plant-derived drugs in the upcoming years (Kumar and Gupta, 2008).

From the medical point of view, the most important plant constituents are the pharmacologically active compounds, including flavonoids, alkaloids, tannins, glycosides, sugars, and organic acids. However, medicinal plants contain considerable amounts of mineral constituents, and the presence of essential elements is a prerequisite for correct plant growth and development (Weber and Koniecznyński, 2003).

There are already publications dealing with total element concentrations in medicinal plants, but only a few investigations deal with more detailed information on the forms of these elements, for example, availability of metals by using different extractants, or correlation of extracted metals with total amounts of organic substances (Zimna and Piekos, 1988; Szentmihályi and Then, 2000).

Water availability is one of the most limiting factors in crop production and productivity. Over the past decade, the increased use of irrigation water and the concern over groundwater resources has brought about awareness to efficiently use water resources (Chai *et al.*, 2005; Molassiotis *et al.*, 2006). In dryland cropping systems, improving water use efficiency is an important goal for crop management and improvement. Maximizing available soil moisture and improving the crop's ability to sustain growth during water deficit periods will contribute to improved productivity and water use efficiency. By reducing root growth, zinc deficiency can limit the crop's ability to use soil moisture reserves (Nable and Webb, 1993), but the effect of Zn nutrition on the water stress response and plant water relationships has received little attention (Khan *et al.*, 2004). Molassiotis *et al.* (2006) have reported Fe decrease in response to water stress. However, water deficit did not significantly influence Zn content in cherry rootstock Gisela (Sivritepe *et al.*, 2008). The content of Fe and Zn in leaf tissue of *Chlorophytum borivilianum* was increased by both water deficit and waterlogging stresses. On the other hand, an increase in Fe content was observed in tuber tissue under both kinds of stresses (Nikam and Chavan, 2009).

We currently have little information on Fe and Zn uptake by chamomile plants under different water stress conditions. Therefore, we assessed changes of these metals in chamomile leaves under water deficit conditions.

MATERIALS AND METHODS

Seeds of *Matricaria chamomilla* L. tetraploid var. Bodegold, a chamazulene-high mixed type with a high bisabolol content obtained in Germany were used in the present study. Seeds were sown in 10 L pots filled with soil. The soil texture was clay-loam (28% silt, 33% clay, and 40% sand) with 22.5% field capacity, 1.54 g cm⁻³ soil density, 1.98% organic matter, and pH 7.6. Greenhouse temperature and relative humidity were 25 ± 3 °C and 55

± 3%, respectively, during the growing season. Pots were placed in a glasshouse and irrigated regularly until four true leaves (two cotyledons + four leaves) appeared and were fully grown. Treatments were then carried out (24 d after sowing, DAS).

Two different experiments were conducted to evaluate the effect of water deficiency on Fe and Zn uptake by German chamomile. In Experiment 1, treatments were arranged in four irrigation regimes (i.e., 95%, 80%, 65%, and 50% field capacity determined gravimetrically) based on a randomized complete block design with five replicates (10 plants per replicate). The plant watering interval was calculated daily. Pots for each treatment were weighed to refill water in order to reach the required moisture level in the pots at their respective stress levels. Irrigation regimes lasted 100 d.

In Experiment 2, irrigation disruptions were applied during five different growth stages of German chamomile based on a randomized complete block design with four replicates (10 plants per replicate). Irrigation treatments consisted in no irrigation during stem formation (43 DAS), flower onset (65 DAS), full bloom (72 DAS), first harvest (76 DAS), and second harvest (90 DAS) as compared with a fully-irrigated control based on the phenology of *Matricaria chamomilla* L. (Pirzad *et al.*, 2010). During the rest of the season, plants were irrigated at 100% field capacity (FC) the same as the control plants when soil moisture content decreased to 80% FC.

Fully expanded leaves were harvested and air-dried at 85 °C to determine Fe and Zn. One gram of dried and finely ground plant material was transferred to a porcelain crucible and combusted in a muffle furnace with an inner volume of 5 L at 470 °C for 12 h. After cooling to room temperature, 10 mL 4 M HNO₃ were added to dissolve the remaining ash. Two hours later, 40 mL of deionised water were added and the samples filtered through MN 614 filter into polyethylene bottles (Schnug and Haneklaus, 1997). Insoluble residues were relatively quantified after ashing by estimating the area of the filter papers covered with soot by the MOCHA image processing system (Rundel, 1992). Both Fe and Zn were analyzed by atomic absorption spectroscopy (AAS) employing atomization in an air/acetylene flame (Schnug, 1982).

Statistical analyses were performed with the MSTATC software. Comparison of means was by Student-Neuman-Keul's Test (SNK) at P < 0.05.

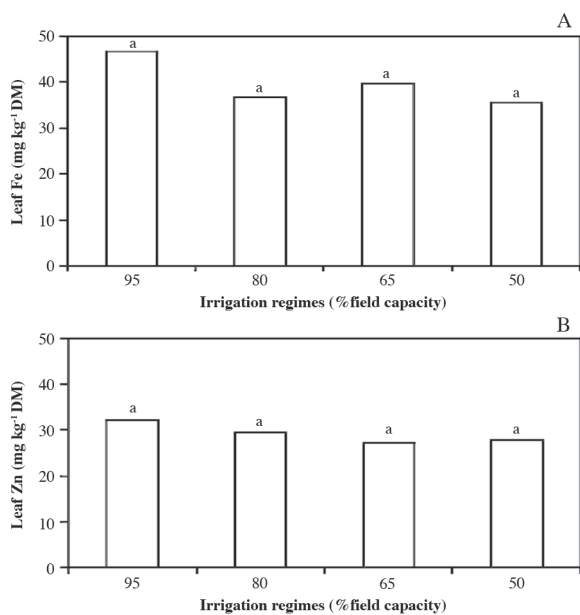
RESULTS AND DISCUSSION

In Experiment 1, irrigation regimes have no significant effect on Fe and Zn uptake by German chamomile (Table 1). Despite the nonsignificant effect, total mean Fe (39.68 mg kg⁻¹) and Zn (29.15 mg kg⁻¹) were at high levels in the plants (Grejtovský *et al.*, 2006). It seems that severe stress (excessive water/water deficit) is needed to reduce Fe and Zn uptake by German chamomile (Figure 1).

Table 1. ANOVA results for the effect of irrigation regimes on Fe and Zn uptake in *Matricaria chamomilla* L.

Source of variation	Experiment 1			Experiment 2		
	df	Fe	Zn	df	Fe	Zn
Replicates	4	0.003 ^{ns}	0.014 ^{ns}	3	0.004 ^{ns}	0.005 ^{ns}
Irrigation regimes	3	0.006 ^{ns}	0.003 ^{ns}	5	0.253 ^{**}	0.219 ^{**}
Error	12	0.009	0.012	15	0.013	0.064
CV, %		13.89	17.16		8.79	13.95

ns, *, **: non significant and significant at 5% and 1% probability levels, respectively. df: degrees of freedom; CV: coefficient of variation.



The same letters over the bars indicate non significant differences ($P \leq 0.05$).

Figure 1. Comparison of means of Fe (A) and Zn (B) uptake under different irrigation regimes in Experiment 1.

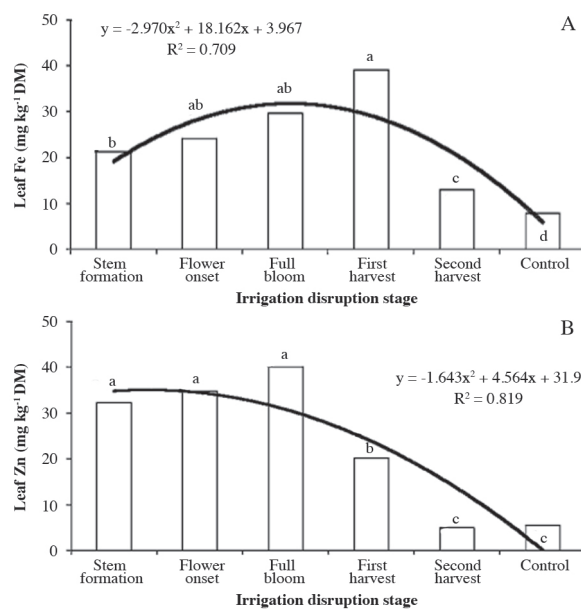
In Experiment 2, the effect of irrigation regimes on Fe and Zn uptake was highly significant ($P \leq 0.01$) (Table 1). Comparisons of means indicated that the highest amount of Fe (39.0 mg kg^{-1}) was obtained from irrigation disruption at first harvest. This value was similar to Fe uptake observed in irrigation disruption at flower onset (24.23 mg kg^{-1}) and full bloom (29.65 mg kg^{-1}). Early and late irrigation disruption significantly reduced Fe uptake. Therefore, irrigation disruption in stem formation (21.3 mg kg^{-1}), second harvest (12.95 mg kg^{-1}), and the control (7.79 mg kg^{-1}) had a decreasing trend of Fe uptake (Figure 2A). Regression between Fe uptake and irrigation showed a binomial function that had an increasing trend for the latest irrigation disruption for plants with maximum irrigation until the flowering stage. Irrigation continued after the flowering stage and caused reduced Fe uptake by chamomile plants; therefore, the control treatments (irrigation throughout the life cycle) had the lowest Fe uptake (Figure 2A).

Zinc uptake had a maximum value (40.0 mg kg^{-1}) in irrigation disruption at the full boom stage, which is similar to Zn uptake in irrigation disruption at flower

onset (34.75 mg kg^{-1}) and stem formation (32.25 mg kg^{-1}). However, later irrigation disruptions at first and second harvest caused lower Zn uptake by chamomile. Therefore, the lowest values of Zn were obtained from irrigation disruption at second harvest (5.0 mg kg^{-1}) and the control (5.5 mg kg^{-1}) (Figure 2B). Regression between Zn uptake and irrigation was also binomial. This function led to a maximum Zn uptake in irrigation disruption at full bloom (midway stage of life cycle), and regular irrigation clearly reduced Zn uptake by chamomile plants (Figure 2B).

Zn is highly mobile in the soil, is easily taken up by plants, and can thus accumulate in the biomass to extremely high phytotoxic concentrations (Vysloužilová *et al.*, 2003). The accumulation rate depends on soil conditions, plant species, genotype, climate, and agronomic practice (Tlustoš *et al.*, 2001).

According to the observations, the commercial chamomile drug sold in pharmacies contains approximately $20\text{--}50 \text{ mg Zn kg}^{-1} \text{ DM}$ (Grejtovský *et al.*, 2006). Comparable values ($30\text{--}50 \text{ mg kg}^{-1} \text{ DM}$) were reported by Chizzola (1989) in chamomile antheridia samples obtained from growers in Austria. Among the potentially toxic elements usually studied, Zn belongs to the most mobile trace elements with high bioavailability and high plant transfer. The maximum Zn concentration in chamomile shoots DM (271.0 mg kg^{-1}) most likely did not reach the phytotoxic level since the plants did not show any signs of chlorosis or any visual symptoms indicating excessive Zn. On the other hand, Kabata-Pendias and Pendias (1992) already assumed potential phytotoxic effects at Zn concentrations exceeding 100 mg kg^{-1} plant DM.



The same letters over the bars indicate non significant differences ($P \leq 0.05$).

Figure 2. Comparison of means of Fe (A) and Zn (B) uptake of leaves under different irrigation regimes and their regression in Experiment 2.

CONCLUSIONS

In accordance with research objectives, our findings indicated that Fe and Zn uptake were not affected by irrigation distances, but they were significantly affected by irrigation regimes as irrigation disruption at different growth stages. Comparisons of means and binomial function between Fe uptake and irrigation showed an increasing trend for the latest irrigation disruption to flowering stage. However, excess water, irrigation continued after the flowering stage reduced Fe uptake, so that irrigation throughout the life cycle had the lowest Fe uptake. The binomial regression between Zn uptake and irrigation showed a maximum Zn uptake in irrigation disruption at full bloom (midway stage of life cycle), and regular irrigation clearly reduced Zn uptake by chamomile plants. Finally, excess water reduced Zn uptake, but both excess water and strong water deficit reduced Fe uptake.

Influencia del déficit hídrico en la absorción de hierro y zinc por *Matricaria chamomilla* L.

La deficiencia de hierro y zinc es un factor limitante de la producción con grandes implicaciones para producción de cultivo en terreno en muchas regiones agrícolas del mundo como fuentes esenciales de Fe y Zn en dietas para humanos y animales. Para evaluar captación de Fe y Zn por manzanilla (*Matricaria chamomilla* L.) cultivada en varias condiciones de sequía, se realizaron dos experimentos bajo condiciones de invernadero. Los tratamientos fueron distribuidos en cuatro regímenes de riego (95, 80, 65, y 50% capacidad de campo) con cinco repeticiones y seis interrupciones del riego (formación de tallo, inicio de floración, floración completa, primera cosecha, segunda cosecha, y sin interrupción) con cuatro repeticiones basadas en diseño de bloques completos al azar en Experimentos 1 y 2, respectivamente. En Experimento 1, los regímenes de riego no tuvieron efecto significativo en captación de Fe y Zn por la manzanilla alcanzando una media total de Fe (39.68 mg kg⁻¹) y Zn (29.15 mg kg⁻¹). En Experimento 2, la más alta cantidad de Fe (39.0 mg kg⁻¹) se obtuvo de interrupción de riego en primera cosecha, similar a los resultados de interrupción de riego en inicio de floración y floración completa. Pero, la interrupción del riego en formación de tallo (21.3 mg kg⁻¹), segunda cosecha (12.95 mg kg⁻¹) y control (7.79 mg kg⁻¹) tuvo una tendencia a reducir la captación de Fe. El valor máximo de Zn (40.0 mg kg⁻¹) se obtuvo de interrupción del riego en estado de floración completa, lo cual también fue similar a la interrupción en inicio de floración y formación de tallo. Sin embargo, interrupciones de riego posteriores, en primera y segunda cosechas redujeron la captación de Zn en manzanilla. Por lo tanto, los menores valores de Zn se obtuvieron con interrupción de riego en segunda cosecha (5.0 mg kg⁻¹) y control (5.5 mg kg⁻¹). Las regresiones entre captación de Fe o Zn y riego mostraron una función binomial.

Plants take up Cd, which is non-essential and potentially toxic for higher plants, animals, and humans, and it is easily accumulated in edible plant parts. From this point of view, proper attention should be paid to chamomile plants which accumulate Cd (Grejtovský and Pirč, 2000; Grejtovský *et al.*, 2001). Adding Zn to the soil at a rate of 50 mg kg⁻¹ decreased Cd concentration in shoots by 10% and in anthodia by 37%. An additional increase in soil Zn concentration caused a decrease in Cd accumulation in chamomile shoots by 18% at a rate of 300 mg Zn kg⁻¹ soil (Grejtovský *et al.*, 2006).

Measurements over longer periods of time did not improve the correlation so that accurate assessment could be done in a relatively short time period (Barton and Abadía, 2006). This fully agrees with our results of Zn in chamomile plants.

Increasing drainage in low-lying areas can minimize compaction and improve Fe availability due to both improved oxygen/moisture ratio and general root growth improvement (Tisdale *et al.*, 1993). The opposite effect is less frequently observed with the severity of Fe chlorosis, although less in the poorly drained soil as compared with normal areas. Possible explanations include reduced water stress in drought conditions, soil salt dilution, and/or increase in Fe reduction under anaerobic conditions (Franzen and Richardson, 2000; Hansen *et al.*, 2003). The bioavailability of Fe increases in soils that are saturated for extended periods. As oxygen is depleted in a saturated soil, Fe³⁺ is reduced to the most available Fe²⁺ form for the plant. As the soil dries and the oxygen concentration increases, Fe is oxidized to the insoluble ferric form. Field crops other than rice have reduced root growth and eventually die under oxygen deprivation in the root zone (El-Shatnawi and Makhadmeh, 2001). Therefore, Fe chlorosis can be reduced for plants growing in poorly drained soils, but the effect may be negligible or opposite if plant root growth and biochemistry are inhibited by oxygen deficiency.

Although conflicting effects for Fe nutrition are observed under poorly and well-drained soil conditions, it is generally advisable to implement cultural practices with good water drainage. In addition to drainage, modifying or managing irrigation water can impact the development of Fe deficiency. High carbonate/bicarbonate concentration in soil water exacerbates Fe chlorosis (Inskip and Bloom, 1987; Hansen *et al.*, 2003). Drainage should reduce soil bicarbonate formation in rainfed areas. Under irrigation conditions, acidifying irrigation water reduces bicarbonate concentration and slowly dissolves soil carbonates, which can result in improved Fe nutrition. Another water management issue is salinity. Accumulation of salts is known to be related to Fe chlorosis. Thus, management of soil salts through proper drainage and irrigation practices can lessen the development of Fe deficiency (Hansen *et al.*, 2003; Siebner-Freibach *et al.*, 2004).

Palabras clave: riego, hierro, *Matricaria chamomilla*, micronutrientes, zinc.

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