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Response of Jatropha on a Clay Soil to Different Concentrations of Micronutrients

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ABSTRACT

In recent years *Jatropha curcas* L. has emerged as a biofuel crop with potential for its production in marginal land with application of treated sewage water. Since this is a new crop for its profitable cultivation, additional research is needed to develop optimal management programs, including macro and micronutrients applications. A pot experiment was conducted in a Greenhouse at the National Research Center, Dokki, Cairo, Egypt, during 2010 summer to evaluate effects of varying concentrations of iron (Fe), manganese (Mn), and zinc (Zn) in irrigation water (0, 50, 100, 150, 200 and 250 ppm) on the growth, biomass production, photosynthetic pigments, and mineral nutrients status in the plants. Increasing concentrations of Fe, Mn, and Zn in irrigation water up to 150 ppm increased the biomass weight, photosynthetic pigments, and nutrient uptake by Jatropha plants. Further increase in concentrations of micronutrients showed negative effects on the above response parameters. Therefore, this study demonstrates that Jatropha can be grown under irrigation using waste water containing reasonable concentrations of micronutrients and heavy metals. This property of Jatropha provides some support for potential use of this crop for phytoremediation of metal contaminated soils. However, long term field research is needed to further verify both the above beneficial effects.

Keywords: Irrigation; Growth; *Jatropha curcas* L.; Mineral nutrition; Photosynthetic Pigments; Heavy Metals; Sewage Sludge

1. Introduction

Egypt is facing fresh water shortage due to rapidly increasing demands of population growth, which is about 2 million per year. The expansion of agricultural production in the desert area to meet the growing demand for food production has further accelerated the increasing pressure on the available fresh water resources. Therefore, there is an interest in using poor quality water for irrigation. One such source of poor quality irrigation water is treated sewage water, which contains large amount of organic materials and some inorganic elements essential for plant growth. But it also may contain non-essential heavy metals which when present in large amount could be harmful if consumed in excess quantities through food chain [1]. Although the use of treated waste water is restricted for irrigation of food crops, this can be a good source for irrigation of non-food crops.

The concentrations of Fe, Mn, and Zn in treated sewage water in different locations in Egypt are in the range of 5100 - 20900, 120 - 476 and 243 - 1200 ppm respectively. Excessive availability of micronutrients, including Zn, Fe, Mn and Cu, in the growing medium can affect normal ionic balance by interfering with the uptake, transport, and osmotic regulation of essential ions. This in turn will disrupt metabolic processes such as transpiration, photosynthesis and enzyme activities related to metabolism. Zinc phytotoxicity also induces oxidative stress by generating free radicals and reactive oxygen species (ROS) [2-4].

Increasing energy consumption worldwide has shifted attention to explore additional sources of energy alternate to fossil fuels. Recently, Jatropha has been investigated mainly as a potential source of oil substitute for diesel. Jatropha can also be used as a green manure, hence can be used as a soil amendment for improving soil properties. The multitude of beneficial uses of this crop [5] is anticipated to result in large scale planting, particularly on somewhat marginal land. This is expected to result in increased employment opportunities both for its production and processing, thus can boost rural economy [6-9]. Substantial expansion in acreage under this crop is necessary to make significant contributions towards lowering the reliance on fossil fuels [10]. Despite growing in-

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terest and advocacy in expanding acreage under Jatropha, very little is known on agronomic management of this crop with respect to optimal management of all inputs to maximize total production and net returns. Jatropha seedlings have demonstrated a high degree of tolerance to Cu concentrations up to 800 ppm in sand culture [11], and tolerance to 400 ppm lead *in vitro* embryo culture [12]. However, the effects of elevated concentrations of multiple micronutrients, such as Zn, Mn and Fe, on the growth of Jatropha have not been studied. Therefore, the aim of the present study was to investigate the effects of different concentrations of Zn, Mn and Fe on the growth of Jatropha seedlings.

2. Materials and Methods

A pot experiment was conducted in a greenhouse during the 2010 summer at the National Research Center (NRC), Dokki, Giza governorate, Egypt. This region is characterized by an arid climate with warm winter and hot summer with average daily temperature ranges from 14˚C to 31˚C. The long-term average rainfall is about 25.7 mm/year, and relative humidity from 50% to 70% [13]. Soil used in the pot experiment was sampled from 0 - 15 cm depth from NRC Experiment Station, Shalkan Kalubia governorate, Egypt. Some physical and chemical properties of the soil used in this study, as described by Cottenie *et al.* [14] are presented in **Table 1**. Eighteen pots of 35 cm in diameter and 50 cm deep were used. Each pot contained 30 kg of air dried clay loam soil and received 60, 80, and 60 g/pot N, P, and K, respectively, using urea, single super phosphate, and muriate of potash. The above N, P, and K rates were equivalent to 115, 154, and 115 kg/ha. Full rates of P and K were applied at pre-planting. Nitrogen was applied in two equal doses, *i.e.* pre-planting and 30 days after seedling emergence. Five seeds were sown per pot. Plants were thinned 21 days after emergence to leave two plants / pot. The treatments included six concentrations of three micronutrients (Mn, Zn, and Fe) in irrigation water at either 0, 50, 100, 150, 200, or 250 ppm using $MnSO_4$ $ZnSO_4·7H_2O$ and $FeSO_4$ reagent grade chemicals. The above treatments were applied three times within the two and a half month growing period, *i.e.* 30, 45 and 60 days after seedling emergence. Irrigation was done to replenish the water deficit when the available soil water was depleted to 60% water holding capacity. The soil water depletion was measured by weighing the pots. A randomized block design was adapted with 3 replications. At end of the two and a half month growing period fully expanded two leaves from the top were sampled for analysis. Plant height, number of green leaves, fresh and dry weights were recorded. The sampled leaves were washed, air-dried, and dried in oven at 70˚C, and ground in a stainless steel mill. Chemical analysis was carried out according to the method described

Table 1. Physical and chemical characteristics[1] of the soil used in the pot experiment.

Particle size distribution:		
Coarse Sand	>200 µm	9.70
Fine Sand	200 - 20µm	16.75
Silt	20 - 2 µm	35.22
Clay	<2 µm	38.33
Soil Texture		Clay loam
Soil Chemical Analysis		
pH	(1:2.5)	7.50
EC ($dS·m^{-1}$)	(1:5)	1.45
$CaCO_3$ (%)		2.65
CEC (C $mole·kg^{-1}$)		30.56
OM	(%)	1.35
Soluble Cations (meq/100 g soil)		
Na^+		1.95
K^+		0.36
Ca^{2+}		2.68
Mg^{2+}		1.57
Soluble Anions (meq/100 g soil)		
HCO_3^-		0.97
Cl^-		1.8
SO_4^{-2}		3.79
Available macro-nutrients (%)		
N		0.49
P		0.27
K		0.93
Available micro-nutrients (ppm)		
Zn		3.5
Fe		5.4
Mn		7.8

[1]Cottenie *et al.* [14]. EC = Electrical conductivity; CEC = Cation exchange capacity; OM = Organic matter content.

by Cottenie *et al.* [14]. Total nitrogen was determined micro Kjeldahl method, and phosphorus was determined calorimetrically at a wave length of 430 nm using spectrophotometer. Potassium, Ca, and Na concentrations were determined using Jenway flame photometer. Concentrations of Mg, Mn, and Fe were measured by using atomic absorption spectrophotometer, IL 157. Chlorophyll a, b and carotenoids contents were determined following the procedure described by Saric *et al.* [15]. The statistical significance of responses were evaluated following the procedure described by Snedecor and Cochran [16].

3. Results and Discussion

3.1. Some Growth Characteristics

Increased concentrations of Mn, Zn, and Fe in irrigation water increased plant height, biomass weight, number of leaves, and leaf area per plant of Jatropha plants (**Table 2**). The above response parameters peaked at 150 to 200 ppm concentrations of micronutrients. With further increase in concentrations to 250 ppm, the above response parameters declined. The results indicate that application of micronutrients was very effective in promoting the growth of Jatropha plants. The above growth promotion

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Table 2. Influence of micronutrients (Mn, Zn, Fe) concentrations in irrigation water on some growth parameters of Jatropha plants, 75 days after emergence (DAE).

Concentrations (ppm)	Plant height (cm)	Leaf number	Fresh weight (gm)	Dry weight (gm)	Leaf area (cm^2)
0	77	20	56	10.2	69
50	90	24	67.5	12.2	87
100	110	33	92.3	16.7	103
150	128	41	115.0	20.8	109
200	118	37	105.0	19.0	98
250	112	40	112.0	20.3	85
LSD ($P \leq 0.05$)	13	5	15	2	10

LSD = Least significant difference. The treatment differences in excess of the LSD for the respective parameter indicate significant differences.

effect was due to the role of micronutrients in enhanced N assimilation. Our results are somewhat contradictory to that of [17], who reported no growth promotion of *Jatropha curcas* plants over 160 days with increased concentrations (1 to 4 mg/l) of Cu and that of Zn (1 to 8 mg/l). The lack of growth promotion in the latter study appears to be due to rather low concentration increments of micro nutrients, unlike that used in our study. Inadequate availabilities of macro- and micro-nutrients to *Jatropha curcas* L. resulted in visual symptoms of nutritional deficiencies [18]. The impact of nutrient deficiencies on biomass production decreased in the order: Ca > Mg > K > N > P > S for macronutrients, and Fe > Cu > Zn > Mn > B for micronutrients [18]. Increasing rates of Zn to *Jatropha curcas* L. influenced Zn concentrations in the fourth leaf of the plant, sampled at 100 days after sowing (DAS) [19]. Similarly, increasing rate of Cu to *Jatropha curcas* L. increased Cu concentrations in the fourth leaf sampled 80, 100 and 120 DAS [20].

Increasing concentrations of micronutrients in irrigation water increased photosynthetic pigments, *i.e*. chlorophyll a, b, and carotenoids (**Table 3**). Accordingly, 150 ppm of Mn, Zn, and Fe treatment was most effective in promoting the synthesis and accumulation of photosynthetic pigments. Heithholt *et al*. [21] concluded that photosynthetic pigments in soybean were greater for all of the $FeSO_4$ treatments, and that no negative response was evident at higher rates of Fe from 30 - 100 ppm. Effects of Zn (0 and 5.0 mg Zn/kg of soil) on photosynthetic rate (PN), and chlorophyll fluorescence in maize leaves (cv. Zhongdan 9409) grown in different soil moisture regimes (40% - 45% and 70% - 75% of soil saturated water content) were investigated [22]. Zn application did not enhance maize plant adaptation to drought stress. The relative water content and the water potential of leaves were not affected by Zn treatment. The PN of drought-stressed plants was not improved by Zn supply. Plant biomass, stomatal conductance, and yields increased with Zn addition in well-watered plants. Kobraee *et al*. [23] concluded that supply of Zn (8 ppm), Mn, (30 ppm), and Fe (8 ppm) with irrigation water to soybean increased photosynthetic pigments which in turn have favorable response to yield and quality. These micronutrients are required in electronic transport reactions and are essential for the biosynthesis pathway of chlorophyll. Ghavri and Singh [24] demonstrated that adequate availability of Fe was essential for chlorophyll biosynthesis and function, energy transfer, and cell metabolism. Iron is a constituent of certain enzymes and proteins required for N fixation and plant respiration.

4. Mineral Status

Concentrations of all mineral elements in Jatropha plant leaves increased with increasing concentrations of Mn, Zn, and Fe up to 150 ppm in irrigation water (**Tables 4** and **5**). The levels of mineral elements in the leaves decreased with further increase in concentration of micronutirents in the irrigation water. At the 150 ppm level of Fe, Mn, and Zn treatment the leaf concentration of N was 143% greater than that for the plants which received no micronutrient supplement (**Table 4**). For the similar comparison the increase in concentration of other macronutrients was in the range of 21% to 42%, while that of Zn, Mn, and Fe were 83%, 75%, and 55%, respectively. The increase in plant biomass weight and concentration of various mineral elements with increasing amounts of Fe, Mn, and Zn in irrigation water (up to 150 ppm) contributed to an increase in total uptake of mineral elements with increasing concentration of micronutrients in the irrigation water up to 150 ppm (**Figures 1** and **2**). The increased concentrations of Zn, Mn and Fe in the irrigation water appeared to influence ionic system which in turn affects the uptake, transport, photosynthesis and enzyme activities related to metabolism. The increased uptake of Fe, Mn, and Zn by Jatropha plants with increased availability of these micronutrients/heavy metals in the root environment appeared to suggest that Jatropha can be a suitable accumulator of heavy metals from soils, thus can be used for phytoremediation of heavy metals contaminated soils. Edaphic factors such as soil pH, low organic matter, soil aeration, high soil phosphorus (P), forms of nitrogen (N) applied, Fe:Zn balance, Fe:Mn

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Table 3. Influence of micronutrients (Mn, Zn, Fe) concentrations in irrigation water on photosynthetic pigments of Jatropha plant leaves, 75 days after emergence (DAE).

Concentrations (ppm)	Chl_a	Chl_b	Chl_a + Chl_b	Carotinoids	Chl_a:Chl_b	Chl_a + Chl_b / Carotinoids
0	3.99	1.67	5.66	2.79	2.39	1.31
50	4.87	1.96	6.83	3.74	2.48	1.82
100	5.42	2.03	7.45	4.60	2.67	1.62
150	6.24	2.47	8.71	6.09	2.97	1.43
200	5.37	2.19	7.56	4.22	2.62	1.79
250	4.63	2.05	6.68	4.22	2.27	1.58
LSD ($P \leq 0.05$)	0.86	0.47	0.62	0.74	0.14	0.24

LSD = Least significant difference. The treatment differences in excess of the LSD for the respective parameter indicate significant differences. Chl_a = Chlorophyll a; Chl_b = Chlorophyll b.

Table 4. Influence of micronutrients (Mn, Zn, Fe) concentrations in irrigation water on concentrations of macronutrients in Jatropha plant leaves 75 days after emergence (DAE).

Mixture concentrations (ppm)	Percent on dry matter basis					
	N	P	K	Na	Ca	Mg
0	2.1	0.14	4.2	1.60	3.6	0.69
50	2.9	0.18	4.7	1.68	3.9	0.73
100	3.8	0.21	5.0	1.88	4.4	0.78
150	5.1	0.25	5.1	2.08	5.1	0.87
200	4.4	0.23	4.2	2.00	3.9	0.84
250	3.5	0.23	4.2	1.63	3.7	0.72
LSD ($P \leq 0.05$)	0.9	0.02	0.2	0.08	0.7	0.05

LSD = Least significant difference.

Table 5. Influence of micronutrients (Mn, Zn, Fe) concentrations in irrigation water on concentrations of Zn, Fe, and Mn in Jatropha plant leaves, 75 days after emergence (DAE).

Concentrations (ppm)	mg/kg on dry matter basis		
	Zn	Fe	Mn
0	64	89	60
50	96	110	85
100	105	152	105
150	110	194	105
200	88	184	100
250	45	141	87
LSD ($P \leq 0.05$)	6	11	8

LSD = Least significant difference. The treatment differences in excess of the LSD for the respective parameter indicate significant differences.

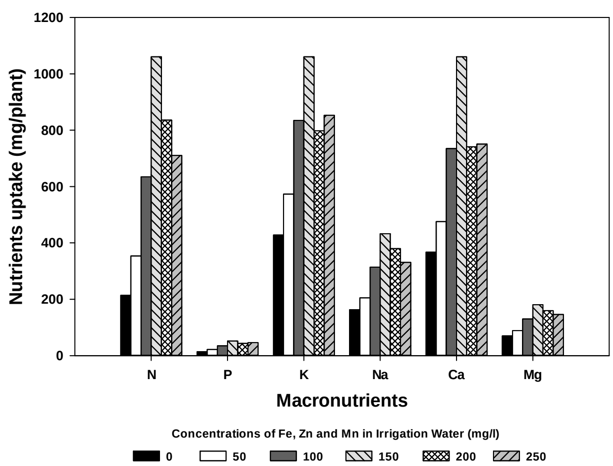

Figure 1. Effects of different concentrationsof Fe, Mn, and Zn in irrigation water on total amounts of macronutrients in Jatropha plants.

balance, K:Fe balance, can affect the availability of Fe to the plants [24]. Different plant species develop adaptive mechanism to survive in soils with very high amounts of Fe and other metals. The results of this study indicate that Jatropha is suitable plant species for phytoremediation of Fe-contaminated soils. Kumar *et al*. [25] have demonstrated beneficial effects of using dairy manure or biosolids amendments to enhance the growth of Jatropha plants in heavy metal contaminated soils. They also showed enhanced growth of Jatropha plants with Zn amendment. Although this pot experiment has indicated beneficial effects of increased concentrations of micronutrients in

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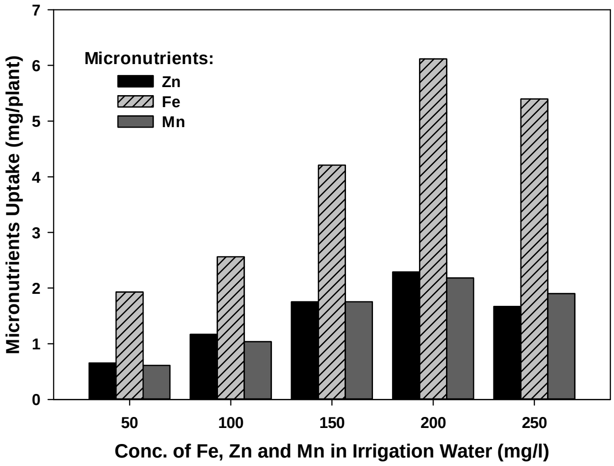

Figure 2. Effects of different concentrations of Fe, Mn, and Zn in irrigation water on total amounts of micronutrients in Jatropha plants.

irrigation water on Jatropha plants growth, photosynthetic pigments, and nutrients uptake, further long-term field experiments are needed to confirm these beneficial responses.

5. Conclusion

This study has demonstrated beneficial responses of Jatropha plants to increased concentrations of Mn, Zn, and Fe in irrigation water up to 150 ppm. No favorable response was evident with further increase in micronutrients concentrations. However further studies are recommended to evaluate the role of micronutrients application to soil vs. foliar on the growth and photosynthetic pigment response of Jatropha in long term field studies under varied soil and climate conditions. With potential increase in production of Jatropha as a biofuel feedstock in the future, developing optimal nutrient management, including micronutrients management, will become a priority to manage this new crop in difficult production regions and utilize resources, which are unsuitable for production of food and feed crops.

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