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Research Article
Remedying Zn Deficiency in Mango Orchards with Foliar Spays

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Abstract
Background and Objectives: Mango (*Mangifera indica* L.) cultivation is new in Saudi Arabia and rigorous fertilization programs are yet to be implemented to help growers deal with the widespread nutrient deficiencies in their orchards. Use of fertilizers especially micro-nutrients is still erratic and very variable from one grower to another. The present study was initiated after Zn deficiency symptoms were discovered in several mango orchards of Jazan region of Saudi Arabia. **Materials and Methods:** The experiment was carried out in two orchards using two Zn products (Zn sulfate and chelated Zn). Mature mango trees of the cultivar 'Sensation' on 'Sudani' rootstock were sprayed either immediately before bloom or both before and after bloom. **Results:** The application of zinc did increase leaf zinc concentrations and improved net photosynthetic assimilation rates. The deficiency symptoms decreased but did not disappear completely possibly because of other nutrients being deficient too, especially Cu which concentration was below the optimum range. To deal with the wide-spread nutrient deficiency symptoms due to soil alkalinity and lack of consistent fertilization, growers need to spray regularly with micro nutrients and to adjust soil pH which was too high. **Conclusion:** This study reports for the first time about the widespread micro nutrient deficiencies in Mango orchards in Jazan. It should be emphasized that making fertilization decisions based on visual symptoms alone can be risky when multiple essential elements are deficient. In the present case, there were clear indications of Zn deficiency but the analyses showed that Cu is more of a problem.

Key words: Mango, mineral nutrients, zinc, copper, soil salinity, deficiency symptoms

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mango (*Mangifera indica* L.) cultivation is new to Saudi Arabia and adequate fertilization programs are yet to be adopted by growers. A recent survey of mango orchards in the southern region of Jazan revealed a rather limited use of fertilizers. More often than not growers would limit their fertilization to the application of livestock manure and a source of nitrogen in the form of urea or di-ammonium phosphate (DAP). The importance of micro nutrients is often overlooked by these growers. In several orchards in the area, the trees had witch-broom-like shoots indicative of possible Zinc deficiency (Fig. 1). Zinc deficiency is known to inhibit growth and reduce leaf size[1-3]. The inter nodes become shorter and the leaves are smaller and curled upward in a rosette disposition at the end of the twigs[4]. Furthermore, Zn deficiency also causes mango flower cluster and fruit malformation thus reducing yield and the commercial value of the crop[5].Commonly, the application of Zn along with iron and boron greatly improves the quality of mango fruit[6]. It is well known that Zn deficiency is a major nutritional concern for fruit trees grown on calcareous, saline and sodic soils with high pH[7,8]. Most mango-growing areas of Jazan have alkaline soils and show indications of salinization due to irrigation with high salinity hazard water[9].

Zn is involved in several biochemical processes because of its tendency to form tetrahedral complexes with N-, O- and S-donor ligands[10]. It is an important constituent of key enzymes involved in cellular electron transport[11]. Zinc is essential for the synthesis of the plant hormone auxin because it is required for the synthesis of the amino acid tryptophan which is a precursor for the latter[12]. Zinc is also needed to maintain cell membrane integrity; it preserves the structural orientation of phospholipids and membrane proteins and maintains ion transport systems[13,14].

The purpose of the present study was to test the efficacy of foliar spays of locally available Zinc sources to remedy Zn deficiency symptoms in mango orchards in Jazan region.

MATERIALS AND METHODS

Plant material: The experiment was carried out in two mango orchards during the period from January-May, 2016:

- Orchard 1 is located in the experimental farm of Jazan Agriculture Research Centre, Abou-Areesh, Saudi Arabia (17.02°N; 42.85°E). A block of 23 years old mango trees of the cultivar 'Sensation' on 'Sudani' rootstock was used in this study. The site has a deep sandy-loam soil with a surface (0-30 cm) pH value of 8.0. The trees were spaced 10×10 m. The orchard was clean-cultivated throughout the year, irrigated with drip-emitters and fertilized with DAP, potassium sulfate and occasionally a micro nutrient mix. Five lots of 3 trees each were selected in each of four adjacent rows (20 lots in total). Each row represented a replicate. One tree was left untreated between every two 3-tree lots. In each row, each one of the five lots was randomly assigned to one of five treatments: untreated control (Ctr), one foliar spray with chelated Zinc (Zinc EDTA, 12% Zn) before bloom (Zc1), one foliar spray with Zinc sulfate ($ZnSO_4$, 23% Zn) before bloom (Zs1), two foliar sprays with chelated Zinc one before bloom and another after (Zc2) or two foliar sprays with $ZnSO_4$ one before bloom and another after (Zs2).The treated trees were sprayed to run-off either with a 0.2% (w/v) solution of chelated Zinc or 0.1% (w/v) $ZnSO_4$. The spraying was carried out early in the morning with an air blast sprayer. The first application was carried out on 7 January while the second application was done on 3 March. Bloom started during the third week of January. On 3 March, most flowers were set with fruitlets' diameters measuring around 2 mm
- Orchard 2 is a private propriety located about 10 km from the first one (17.026°N; 42.8°E). A block of 25 years old trees of 'Sensation' on 'Sudani' rootstock was used. The soil is deep and sandy-loam with a pH of 7.8. The trees were spaced 8×8 m. The orchard was clean-cultivated throughout the year and irrigated with drip-emitters. Fertilizers were rarely used here but

Fig. 1: Potential Zn deficiency symptoms on mango trees in Jazan, Saudi Arabia

animal manure was regularly applied. The rest of the experimental setup is similar to the one used in Orchard 1

Leaf samples were taken from both locations on 7 April (about 2 weeks before harvest). The leaves were collected from the three trees of each lot. A sample of about 50 fully developed leaves was taken from the mid-section of non-fruiting branches of the early spring growth flush. The leaves were collected evenly from the perimeter of the tree canopy at a height between 1.5 and 2.5 m. The leaves were immediately taken to the laboratory where they were briefly rinsed with deionized water to remove dust and any pesticide residues then dried in an air-forced oven at 70°C for 72 h. After drying, the leaves were grinded to pass a 0.4 mm-diamter (40-mesh) screen with an Ika laboratory mil then stored in a desiccator until analyzed[15].

Leaf mineral analysis: Sub samples of dry and grinded leaf tissue were ashed in a muffle furnace at 200°C for 2 h then at 500°C for 5 h. The ashed samples were dissolved in 0.1 N nitric acid then analyzed for Zn, Fe, Mn and Cu with an Inductively Coupled Plasma Spectrometer (ICA-AES model ICAP6000Duo; Thermo Scientific, USA) according to Donohue and Aho[16].

Gas exchange measurements: Net photosynthetic assimilation rate (A_{net}) and stomatal conductance (g_s) were measured at both locations on 7 April (about 2 weeks before harvest) on fully expanded leaves (4th-6th leaf from the shoot tip) on actively growing shoots. The middle tree from each lot was used for the measurements (four trees per treatment). Four measurements were made on each tree. The measurements were carried out *in situ* between 9 am and 11 am at incident photo synthetic photon flux density (PPFD) of 1400-1600 μmol m^{-2} sec^{-1} and with an air temperature ranging from 25-31°C. These parameters were measured using a CIRAS-2 Portable Photosynthesis System (PP system, USA).

Leaf chlorophyll concentration: The leaves used for chlorophyll determination were collected from the same shoots used to measure gas exchange. The leaves were collected from the middle of actively growing no fruiting shoots. They were transported to the laboratory in a cool box. Leaf chlorophyll concentration was determined according to Arno[17]. In short, 1 g of fresh leaves were ground to a fine powder in liquid nitrogen in a pre-cooled mortar then homogenized for 30 sec in 5 mL of 95% acetone. The extract was filtered then its absorbance was determined with a spectrophotometer at wavelengths 645 and 663 nm. The following equation was used to calculate the concentration of total chlorophyll:

$$\frac{\text{Total chlorophyll (mg)}}{\text{Fresh leaves (g)}} = (20.2\ (A_{645}) + 8.02\ (A_{663})) \times \frac{V}{W}$$

Where:
A = Absorbance at specific wavelengths
V = Final volume of chlorophyll extract
W = Fresh weight of leaf tissue extracted

Statistical analysis: The data were subjected to a one-way analysis of variance (ANOVA) in a Randomized Complete Block Design with four blocks (each row was considered a block equivalent to a replicate) using OriginPro 2018 software (OriginLab, USA). The independent variable was Zn treatment. Blocking was considered to account for potential differences between rows. Where applicable, the means were separated by Tukey test with a level of significance $p \leq 0.05$.

RESULTS

The 'Sensation' trees had two main flushes of growth, one in late winter-early spring (January-February) and a second one right after harvest in late spring (May). A few vigorous trees had a limited 3rd flush of new growth in September after late summer rains. Flowering occurred in February and fruit maturation started in mid-April which coincides with mango phenology in India[18] but unlike neighboring Egypt and Sudan where fruit maturity starts in June and lasts until mid-fall.

The concentration of Zn in tree leaves was within the optimum range or higher for all treatments at both locations except for the Nil treatment in Orchard 2 where the trees had leaf Zinc levels below the optimum rage but above the deficiency threshold (Table 1). Nevertheless, spraying the trees with Zn-containing solutions did significantly increase leaf Zn levels. This was true in both orchards; one application of chelated Zinc more than doubled the concentration of leaf Zinc whereas one application of Zinc sulfate increased the concentration of Zinc in the leaves by 40% in Orchard 1 and by189% in Orchard 2. The trees that received chelated Zn had considerably higher leaf Zn concentrations than those sprayed with the sulfate form of Zn. The difference was +32 and +24% in Orchard 1 for one application and two applications, respectively. In Orchard 2, the application of chelated zinc increased leaf zinc by 6 and 13% over sulfate zinc application

Table 1: Leaf micro-nutrient concentrations (ppm dry weight) measured in early April, just before harvest, in "Sensation" mango trees in two orchards in Jazan, Saudi Arabia

Treatment[Z]	Zinc source	Number of applications	Zn	Fe	Mn	Cu
Orchard 1						
Ctr	Nil	None	32.0±5[dy]	46.0±3[b]	88.0±6[b]	8.0±1[a]
Zc1	Chelate	1	65.0±4[b]	60.0±1[a]	158.0±25[a]	9.0±2[a]
Zs1	Sulfate	1	49.0±4[c]	52.0±4[b]	123.0±12[a]	9.0±2[a]
Zc2	Chelate	2	89.0±6[a]	54.0±10[ab]	133.0±26[a]	5.0±2[a]
Zs2	Sulfate	2	72.0±5[b]	49.0±2[b]	144.0±10[a]	6.0±2[a]
Orchard 2						
Ctr	Nil	None	18.0±2[c]	19.0±1[b]	38.0±5[a]	2.0±1[b]
Zc1	Chelate	1	55.0±3[b]	20.0±5[b]	40.0±3[a]	2.0±1[b]
Zs1	Sulfate	1	52.0±4[b]	20.0±1[b]	42.0±2[a]	3.0±1[b]
Zc2	Chelate	2	80.0±5[a]	22.0±1[b]	42.0±3[a]	6.0±5[a]
Zs2	Sulfate	2	71.0±4[a]	24.0±1[a]	43.0±3[a]	5.0±1[a]
Adequate conc. range in the leaves[x]			20-40	50-200	50-100	10-50
Deficiency level			<10	<15	<10	<5

[Z]The treatments were: untreated control (Ctr), one foliar spray with chelated Zinc (Zinc EDTA, 12% Zn) before bloom (Zc1), one foliar spray with Zinc sulfate ($ZnSO_4$, 23% Zn) before bloom (Zs1), two foliar sprays with chelated Zinc one before bloom and another after (Zc2) or two foliar sprays with $ZnSO_4$, one before bloom and another after (Zs2). Each value is the mean (±SE) of four replicates of three trees (n = 12). [y]Means within a column followed by the same letter are not significantly different at p≤0,05. [x]According to Quaggio[19]

Table 2: Net photosynthetic assimilation rate (A), stomatal conductance (g_s) and total chlorophyll (Chl) concentration measured in early April, just before harvest, in mature "Sensation" mango trees sprayed with Zn-containing solutions at different concentrations

Treatments	A (μmol m^{-2} sec^{-1})	g_s (mmol m^{-2} sec^{-1})	Chl (mg g^{-1} FW)
Orchard 1			
Ctr	7.4±0.7[bz]	71.0±16[b]	0.82±0.21[b]
Zc1	9.9±1.0[a]	90.0±7[a]	1.25±0.22[a]
Zs1	11.6±1.9[a]	64.0±15[b]	1.23±0.15[a]
Zc2	12.3±2.0[a]	85.0±11[ab]	1.39±0.14[a]
Zs2	10.5±1.3[a]	76.0±13[b]	1.35±0.17[a]
Orchard 2			
Ctr	8.3±0.8[b]	73.0±11[b]	0.74±0.14[b]
Zc1	10.8±1.0[a]	69.0±8[b]	1.12±0.20[a]
Zs1	9.7±1.2[ab]	79.0±9[ab]	1.08±0.19[a]
Zc2	12.0±1.4[a]	93.0±12[a]	1.16±0.13[a]
Zs2	11.5±1.1[a]	86.0±10[ab]	1.14±0.15[a]

[z]Means within a column followed by the same letter are not significantly different at p≤0.05

for one application and two applications, respectively. Also, spraying the trees twice further increased Zn levels (Table 1). In Orchard 1, applying chelated Zn twice increased leaf Zn by 37%, on average, compared to the single application while applying ZnSO4 twice increased leaf Zn by about 47%. In Orchard 2, the increase was 45 and 36%, respectively.

Furthermore, the application of Zn solutions tended to increase the foliar concentrations of Fe, Mn and Cu as well. Leaf Fe concentrations were below the adequate range but above the deficiency level in both orchards (Table 1). In both orchards, application of fertilizer slightly increased Fe leaf concentrations, which became adequate in Orchard 1 but not in Orchard 2. Similarly, Mn concentrations increased slightly in response to Zn solution application and was within the adequate range in Orchard 1 but below that range in Orchard 2.

Cu was overall below the adequate range in both orchards but more so in Orchard 2 where the concentrations were below the critical deficiency level. There wasn't any consistent effect of the application of zinc on leaf Cu levels.

Over all, deficiency symptoms on new leaves decreased but did not disappear completely especially in Orchard 2 where new leaves were still small and in a rosette disposition especially in early fall.

Application of Zn fertilizers generally increased A_{net} and leaf total chlorophyll concentrations in both orchards (Table 2). A_{net} increased by more than 34 and 17% in Orchard 1 and Orchard 2, respectively, especially when two applications were carried out. Stomatal conductance also increased slightly. However, Zn form and the number of applications did not make, generally, a difference for all three parameters.

DISCUSSION

In the orchards used in the present study, the trees did show symptoms reminiscent of Zn deficiency (small leaves curled upward, sticky exudates from the bark of the trunk and branches) but the deficiency of other element could not be ruled out. Indeed, as leaf analysis showed, Zn was not deficient whereas Cu was.

In both orchards, the deficiency symptoms decreased but did not disappear completely especially in Orchard 2 possibly because of other nutrients being deficient too; in fact, Cu concentration was below the optimum range. It appears that in the orchards used in this study, Cu is the cause of little-leaf symptoms seen on the trees especially during the periods of growth flushes (late winter and late spring). Zn and Cu

Fig. 2: Potential Cu deficiency symptoms on the trunk of a mango trees in Jazan, Saudi Arabia

deficiency may be due to a combination of soil alkalinity and large applications of N fertilizers (DAP in the case of Orchard 1 and animal manure in the case of Orchard 2) rather than a soil deficiency. In other locations, especially where soil salinity is a problem such as in southern Jazan, Zn can also be severely deficient in the soil.

Commonly, copper deficiency symptoms appear in young trees or on new shoots of mature trees which received large amounts of N fertilizers[20,21]. Deficient mango trees would have long, tender and "S" shaped shoots with downward curled leaves. The branches would have boil-like eruptions which sometimes exude a gummy substance[22]. During the execution of the present study, mango trees in the orchards of the southern part of Jazan region near Samta and AhadMasarha did show some of the symptoms described above especially long and slender twigs and gummy exudates from the bark of the trunk and large branches (Fig. 2). However, it was difficult to conclude with certainty that Cu was the problem because of other compounding factors. Indeed, the soils of these orchards were affected by salinity with the tree leaves having typical scorched margins. Also, disease-related die-back of branches was reported in the area possibly due to attacks by the long-horned beetle (*Coptopsaedificator*) which also causes sap oozing on branches[23]. Furthermore, it is known that mango demand for Cu is low[22] and fungicides used in disease control have Cu in their formulation[24].

The reduction in photosynthetic A_{net} may be due to restrictions on stomatal opening or CO_2 diffusion through the mesophyll mediated by root-generated hormonal signals or a reduction in the efficiency of enzymes involved in leaf cell photochemistry such as carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO)[11]. In the present study, Zn applications generally increased A_{net} and leaf chlorophyll concentrations in both orchards regardless Zn form and the number of applications. Zinc is a constituent of the enzyme carbonic anhydrase which catalyzes the conversion of CO_2 and H_2O to carbonic acid; the electrons extracted from water will give rise to the ATP and NADPH needed to reduce carbon dioxide to sugars[8]. It is well documented that improved Zn nutrition increases chlorophyll content of mesophyll cells thus improving photosynthesis[8,25]. Similarly, Weisany *et al.*[8] reported that application of zinc to soybean plants on saline soil reduced the damage inflicted by salt as indicated by less electrolyte leakage and a reduced production of malondialdehyde (MDA); it also increased A_{net} and leaf chlorophyll content.

A short term remedy for micronutrient deficiency problem in Jazan mango orchards would be 2-3 foliar sprays of micronutrient fertilizers in spring. The foliar application would, obviously, side-step the problem of soil alkalinity by getting the nutrients directly into the leaves. However, to be effective, the applications have to be made during new growth flushes (in early and late spring) because older mango leaves have a waxy cuticle which impedes solution uptake. Ultimately, lowering the soil pH through the application of strong acids via the irrigation system will give a more durable solution to the problem.

CONCLUSION

To conclude, the application of zinc did increase leaf zinc concentration and improved Anet. The deficiency symptoms decreased but did not disappear completely possibly because of other nutrients being deficient too, especially Cu which concentration was below the optimum range. It appears that in the orchards used in this study, Cu is the cause of little-leaf symptoms seen on the trees especially during the periods of rapid vegetative growth. In other locations especially where soil salinity is a problem, Zn can also be severely deficient. Zn and Cu deficiency may be due to a combination of a soil alkalinity, large application of N fertilizers (DAP in the case of Orchard 1 and animal manure in the case of Orchard 2) rather than a soil deficiency. A short term remedy would be 2-3 foliar sprays of micro nutrients during spring. However, lowering the soil pH through the application of strong acids via the irrigation system would be more effective in solving the problem.

The experiment proves that making fertilization decisions based on visual symptoms alone can be risky when multiple essential elements are deficient. In the present case, there were clear indications of Zn deficiency but the analyses showed that Cu is the more urgent problem.

SIGNIFICANCE STATEMENTS

Micro nutrient deficiency are wide spread in Jazan Orchards especially the small ones. Cu and Zn are the most commonly deficient nutrients. The problem can be easily solved by foliar sprays or by correcting soil pH. Agricultural extension services need to do more to convince growers of the importance of fertilizing their orchards to maintain yield and the quality of the crop.

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