ORIGINAL ARTICLES

Effect of Copper Oxychloride or Foliafeed D on Vegetative Growth, Leaves Physical and Chemical Properties and Yield of Valencia Orange Trees


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ABSTRACT

The present study was carried out during two successive seasons of 2003-2004 and 2004-2005 to study the effect of different levels of copper oxychloride (0, 2.5, 5 and 7.5 g/l) or micro nutrients fertilizer, foliafeed D (0.625 g/l) foliar applications on growth, fruit yield and fruit quality of Valencia orange trees. Generally, the results reveal that all treatments improved most of the studied growth characters, yield and fruit quality through their favourable effects on leaves physical and chemical composition; plant pigments, total sugars, total soluble phenols, total free amino acids, tryptophan and endogenous plant hormones balance of leaves as well as nutritional status (Zn, Cu, Fe and Mn concentrations) of leaves.

Key words: copper oxychloride, foliafeed, yield, fruit quality, Valencia orange.

Introduction

Deficiency of copper in citrus has been described under different names such as die back and wither-tip, ammoniation, red rust and exanthema. The tender, long and pointed twigs of the tree twist to form an S-like structure. The leaves of these twigs are usually long and deeper in color. In acute deficiency of Cu, small leaves are formed on new twigs with mottled shape (Singh, 2000). Copper deficiency, unlike that of other heavy metals not associated with a leaf chlorosis. Abnormally large, dark green leaves give early indication of deficiency. Twigs die back, and multiple buds form near the juncture with live wood. The most ratable symptoms for identification of copper deficiency are gum pockets under the green bark of young wood and brownish excrescences on twigs and leaves (Bennett, 1993). Common disorders due to copper deficiency are generally stunted growth, distortion of young leaves and, particularly in citrus trees, a loss of young leaves referred to as “summer dieback” (Hopkins and Hiiner, 2004). Thus, the present work was designed to study the effect of copper oxychloride (0, 2.5, 5 and 7.5 g/l) or foliafeed D (0.625 g/l) foliar applications on growth, yield of Valencia orange trees. Also to correct the micronutrients deficiencies which in turn improve the nutritional status, growth, fruiting and fruit quality and consequently decrease and correcting mottle leaf, little leaf and dieback of new twigs of Valencia orange trees.

Materials and Methods

The present study was carried out during two successive seasons of 2003-2004 and 2004-2005 in citrus orchard of El-Kassasin Horticulture Research Station, Ismailia Governorate, Agricultural Research Center, Ministry of Agriculture, Egypt.

Valencia orange trees (Citrus sinensis L. Osbek) budded on Sour orange rootstock (Citrus aurantium) on 12-year-old grown on sandy loam soil at 5 × 5 m were used in this experiment.

Representative soil samples were collected from the Orchard under investigation from three depths (0-30, 30-60 and 60-90 cm) in each block and subjected to the determination of soil-reaction (pH). The initial soil samples were analyzed for physical and chemical characteristics [illustrated in Table 1] as follows:

1. Mechanical analysis was carried out by pipette method, according to Piper (1950).
2. Total calcium carbonate was determined volumetrically using Collin's calcimeter, following Piper (1950).
3. Soil pH was measured in (1:2.5) soil-water suspension using electrode pH-meter, according to Jackson (1973).
4. Soluble cations: Ca$^{2+}$ and Mg$^{2+}$ were measured by versenate method, while Na$^+$ and K$^+$ were measured using flame photometer; according to Richards (1954).
5. Available K was extracted with "NH₄HCO₃-DTPA" according to Soltanpour (1985) and measured flame photometer.

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6. Electrical conductivity (expressed in d/Sm\(^{-1}\)) as well as total soluble ions were measured using electrical conductivity bridge, in the extract of saturated soil past as outlined by Jackson (1973).

7. Soluble anions \([\text{CO}_3^{2-}, \text{HCO}_3^{-}, \text{Cl}^{-}, \text{SO}_4^{2-}]\) were determined titrimetrically according to Richards (1954).

8. Available N was determined using microkjeldahl apparatus, after extracting with 2N KCl solution as outlined by Jackson (1973).

9. Available P and microelements \([\text{Fe}, \text{Mn}, \text{Zn and Cu}]\) were extracted with "NH\(_4\)HCO\(_3\)-DTPA" according to Soltanpour (1985) and measured using spectrophotometer and atomic respectively.

### Table 1: Mechanical and chemical analysis of the orchard soil under experimental trees.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>0-30</th>
<th>30-60</th>
<th>60-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC mmhos/cm at 25°C</td>
<td>3.65</td>
<td>2.86</td>
<td>2.96</td>
</tr>
<tr>
<td>pH</td>
<td>7.90</td>
<td>8.00</td>
<td>8.00</td>
</tr>
</tbody>
</table>

**Particle size distribution:**

<table>
<thead>
<tr>
<th></th>
<th>Coarsed sand</th>
<th>Fine sand</th>
<th>Silt</th>
<th>Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37.20</td>
<td>38.40</td>
<td>14.60</td>
<td>9.80</td>
</tr>
</tbody>
</table>

**Textural class:**

<table>
<thead>
<tr>
<th></th>
<th>sandy loam</th>
<th>sandy loam</th>
<th>sandy loam</th>
</tr>
</thead>
</table>

**Soluble ions (meq/l):**

<table>
<thead>
<tr>
<th>Cations</th>
<th>Ca(^{2+})</th>
<th>Mg(^{2+})</th>
<th>Na(^+)</th>
<th>K(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.00</td>
<td>10.00</td>
<td>8.90</td>
<td>5.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anions</th>
<th>CO(_3^{2-})</th>
<th>HCO(_3^{-})</th>
<th>Cl(^-)</th>
<th>SO(_4^{2-})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.00</td>
<td>4.00</td>
<td>22.00</td>
<td>18.40</td>
</tr>
</tbody>
</table>

**Available nutrient (ppm):**

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>K</th>
<th>Mn</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.30</td>
<td>317.50</td>
<td>6.00</td>
<td>8.00</td>
<td>2.20</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The trees were under drip irrigation system and received 3500 m\(^3\) of irrigation water/fed/year. The uniform fertilization program was as follows: organic manure at 20 m\(^3\)/fed in January. Calcium superphosphate (15.5% P\(_2\)O\(_5\)) at 100 kg/fed mixed with soil and organic manure in two ditches near the roots and under 75% of tree canopy. Nitrogen fertilizer (ammonium nitrate 33.5%) at the rate of 500 g N/tree/year from the beginning mid-February to mid-October. Potassium sulphate (48-52% K\(_2\)O) at the rate of 500 g K\(_2\)O/tree/year from the first of March to the end of September. Copper oxychloride (at the rate of 5 g/l water) were applied as foliar spray with mineral oil on the third week of February. This treatment saved as control.

In addition, pests were controlled according to the recommendations of the Agricultural Ministry.

In the two experimental seasons, the treatments were as follows:

1. Control.
2. As control treatment but without copper oxychloride (zero Cu).
3. As control treatment but with the half dose only of copper oxychloride (1.5 kg/600 l/fed; 2.5 g/l Cu).
4. As control treatment but with the one and half dose only of copper oxychloride (4.5 kg/600 l/fed; 7.5 g/l Cu).
5. Replacement the micronutrients (control) with foliafeed D\(^\circ\) at the rate of 0.625 g per liter, foliafeed D containing 16.52% micronutrients as follows: iron 3% and zinc 7% in the chelated form on EDTA as well as manganese 5%, copper 0.5%, boron 0.5%, molybedinum 0.02% and magnesium 0.5% in the mineral forms.

These treatments were applied as foliar sprays on the third week of February (flower initiation or prebloom stage), the first week of May (cell division of fruitlets stage) and the third week of July (cell expansion of fruit) except 2, 3 and 4 treatments which were applied as foliar sprays on the second week of February and the first week of July.

A complete randomized block design was used, each treatment was replicated three times with one tree for each replicate, thus, the total number of trees in this experiment was 15 (5 treatments \(\times\) 3 replicate).

The following growth characters were recorded of new develop twigs from bud: out growth were used to study: 1) Shoot height (cm), 2) Shoot diameter (cm), 3) Number of leaves/shoot, 4) Leaf area (cm\(^2\)) which estimated by leaf area meter (model CL-203 area meter CID, Inc, USA).
Fresh leaves were extracted with dimethyl Formamid to determine total chlorophylls according to Nornai (1982). Ethanol extract of fresh leaves was used for the determination of total sugars, total free amino acids and total soluble phenols. Determination of total sugars was carried out by using the phenol-sulphuric acid method according to Dubois et al. (1956). Total free amino acids were determined by using ninhydrin reagent according to Moore and Stein (1954). Total soluble phenols were estimated using the folin-ciocalteau colorimetric method as described by Swain and Hillis (1959).

Methanol extract was used to determine gibberellic acid (GA₃), abscisic acid (ABA) and indole acetic acid (IAA) in fresh leaves by Gas Liquid Chromatography according to the method described by Vogel (1975).

In dry matter of leaves, tryptophan was determined by using p-dimethylaminobenzaldehyde according to Spies and Chambers (1949).

In dry matter of leaves, Fe, Mn, Zn, Cu, were determined. Digestion of plant materials were carried out using sulphuric and perchloric acids as described by Piper (1950). Determination of micronutrients (Fe, Mn, Zn and Cu) concentrations were determined by the atomic absorption spectrophotometer (Thermo JarrellsH, AA SCANI).

Fruits yield (weight "kg" and number of fruits/tree) were determined at harvesting time (the first week of February). At harvest stage, a representative sample of 10 fruits as a replicate, with three replicates for each treatment: Average fruit weight (g) and average fruit size (cm³) was measured by water displacement in graduate jar.

Statistical Analysis:

A complete randomized block design was used. The obtained data were subjected to the analysis of variance according to Snedecor and Cochran (1972). Mean between treatments were compared using the L.S.D. values at 0.05 level.

Results and Discussion

1- Vegetative Growth:

1-1-Effect Of Copper Oxychloride:

The results in Table (2) revealed that, most of the studied shoot characters were pronounced increases under the lowest (0 or 2.5 g) or the highest (7.5 g) level of copper oxychloride when compared with those sprayed with the middle level (5 g), as a control treatment.

These results are in agreement with those obtained by Ali et al. (1992) on Citrus sinensis, Alva et al. (1995) on Hamlin orange, while these results were disagreement with Alva et al. (1999) on citrus seedlings.

In this respect, Fry et al. (2001) found that, promotion of polysaccharide scission by Cu-generated apoplastic OH radicals would be likely to contribute to wall loosening, and thus cell expansion, in vitro. These considerations may explain by some of the following observations:

1. Exogenous ascorbate, which converts Cu²⁺ to Cu⁺, can promote cell expansion, e.g. in onion roots, especially under condition favouring ascorbate oxidation (Hidalgo et al., 1991).
2. In soybean hypocotyls, dithiothreitol inhibits both NADH oxidase activity (which forms apoplastic O₂, another agent that converts Cu²⁺ to Cu⁺) and auxin-stimulated cell extension (Morre et al., 1995).

Table 2: Effect of copper oxychloride and foliafeed D on growth characters of Valencia orange trees during 2003 and 2004 seasons.

<table>
<thead>
<tr>
<th>Growth characters</th>
<th>Shoot length (cm)</th>
<th>Shoot diameter (cm)</th>
<th>Leaves number / shoot</th>
<th>Leaf area (cm²) / shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>1st  season</td>
<td>2nd season</td>
<td>1st  season</td>
<td>2nd season</td>
</tr>
<tr>
<td>Control</td>
<td>6.36 7.00</td>
<td>0.165 0.167</td>
<td>9.53 5.60</td>
<td>15.42 10.30</td>
</tr>
<tr>
<td>Cont. zero copper oxychloride</td>
<td>7.79 6.96</td>
<td>0.162 0.174</td>
<td>10.23 7.85</td>
<td>13.69 12.66</td>
</tr>
<tr>
<td>2.5 g/l copper oxychloride</td>
<td>7.70 7.90</td>
<td>0.174 0.172</td>
<td>10.67 6.27</td>
<td>17.76 11.70</td>
</tr>
<tr>
<td>7.5 g/l copper oxychloride</td>
<td>7.70 8.65</td>
<td>0.180 0.178</td>
<td>10.70 6.17</td>
<td>16.25 11.68</td>
</tr>
<tr>
<td>Foliafeed D (0.625 g/l)</td>
<td>8.71 10.00</td>
<td>0.168 0.161</td>
<td>10.23 6.93</td>
<td>14.76 11.90</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>1.97 0.49</td>
<td>0.01 N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Bennett (1993) and Marschner (1995) reported that, Cu is a constituent of the chloroplasts bound to plastocyanine, a component of electron transport chain linking the two photochemical system of photosynthesis. Copper may play a part in the synthesis or stability of chlorophyll. Copper participates in both protein and
carbohydrate metabolism. Copper is a factor in DNA and RNA synthesis, since low levels of DNA are observed in Cu-deficient tissues. There is a specific requirement for Cu in symbiotic N-fixation.

1-2- Effect of foliafeed D:

The results revealed that, in the two successive seasons, significant increases in shoot length, as well as non-significant increases on shoot diameter, leaves number and single leaf area were detected in the shoots of Valencia orange trees treated with foliafeed D when compared with control treatment.

These results are in agreement with those obtained by El-Shazly et al. (2000) as well as Gendiah and Hagagy (2000) on Washington navel orange trees.

In this respect, many investigators suggested that, the favourable effects induce by micronutrients on plant growth might be through its effects on enzyme systems responsible in many metabolic reactions occurs in the plants. In this connection, it can be assumed that, these results might be attributed to the effect of these micronutrients on the internode elongation by increasing plasticity of cell wall through its effect on activated IAA oxidases.

In this respect, Beffa et al. (1990) found that, in maize roots, increasing levels of MnCl₂ were shown to stimulate IAA oxidase activity in vitro. Moreover, Mn plays an important role in the oxidation-reduction processes in the photosynthetic electron transport system. It is essential in the photosystem II for photolysis (Wilkinson, 1994).

Furthermore, Jones et al. (1991) found that, Fe is an important component in many enzyme systems, such as cytochrome oxidase [electron transport and cytochrome (terminal respiration)]. Moreover, Fe functions as a catalyst or part of an enzyme system associated with chlorophyll formation. Leidi et al. (1986) working on soybean, found that catalase activity was increased with the increase of Fe supply chelated form (EDTA). El-Shazly et al. (2000) mentioned that, the high shoot length and leaf area were attained with Fe-EDDHA at 7.5 g/tree through four equal doses in both experimental seasons. Obviously the increase of chlorophyll in the leaves of trees treated with Fe-EDDHA, and therefore their high ability to manufacture sufficient amounts of food materials necessary for supporting the various growth processes, might provide a reasonable explanation for the positive effect of Fe-EDDHA application on the growth of Washington navel orange trees. Bennett (1993) working on Citrus, reported that boron is involved in the transport of sugars across cell membranes and in the synthesis of cell wall material. It influences transpiration through the control of sugar and starch formation. It also influences cell development and elongation. Boron deficiency apparently leads to build up of indole acetic acid (IAA) by blocking IAA oxidase in roots, which inhibits elongation growth. Banik and Sen (1997) reported that, boron is known to play a key role in the extension of plant cell walls through its association with cell wall pectins and specifically in the formation of rhamnogalacturonan-B dimers. Hansch and Mendel (2009) reported that, micronutrients are involved in virtually all metabolic and cellular functions, like energy metabolism, primary and secondary metabolism, cell protection, gene regulation, hormone perception, signal transduction, and reproduction among others.

3- Organic Components:

3-1- Plant Pigments:

3-1-1- Effect Of Copper Oxychloride:

The data revealed that, copper oxychloride has no constant trend could be recorded effect on total chlorophyll of Valencia orange leaves sprayed with either the lowest level of copper oxychloride foliar applications (2.5 g/l) or the highest level (7.5 g/l) compared with zero copper oxychloride treatment or with trees treated with the middle level of copper oxychloride (5 g/l). The highest value of chlorophylls concentration were detected by the leaves of the plants treated with the lowest level of copper oxychloride (2.5 g/l), while the lowest value of chlorophylls were obtained by those treated with the highest level of copper oxychloride (7.5g/l).

In this respect, Boardman (1975) revealed that, Cu is a constituent of the chloroplast protein plastocyanin which forms part of the electron transport chain linking the two photochemical systems of photosynthesis. Bergmann (1992) reported that, Cu⁺ ions are believed to act as a chlorophyll stabilizer, protecting the chlorophyll-protein lipid complex and chlorophyll from premature degeneration. Wilkinson (1994) reported that, more than half the Cu found in plants is localized in the chloroplasts bound to plastocyanine, a component of the electron transport chain linking the two photochemical system of photosynthesis. Copper may play a part in the synthesis or stability of chlorophyll. Purohit (2007) reported that, the chloroplasts contains a copper containing protein called plastocyanin that is essential as an electron carrier in photosynthesis. Spiegel- Roy and Goldschmidt (2008) mentioned that, copper is part of the oxidation- reduction systems, such as ascorbic acid oxidases. It is part of plastocyanin (chloroplast enzyme).
Table 3: Effect of copper oxychloride and foliafeed D on plant pigment (mg/g f.w.) and organic composition (total sugars, total soluble phenols and total free amino acids (mg/g f.w.) as well as tryptophan (mg/g d.w.) concentrations) of Valencia orange leaves in season 2004.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total chlorophyll</th>
<th>Total Sugars</th>
<th>Soluble phenols</th>
<th>Total free amino acids</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.835</td>
<td>2.800</td>
<td>1.880</td>
<td>1.952</td>
<td>0.049</td>
</tr>
<tr>
<td>Cont. zero copper oxychloride</td>
<td>1.791</td>
<td>4.560</td>
<td>3.120</td>
<td>1.376</td>
<td>0.056</td>
</tr>
<tr>
<td>2.5 g/l copper oxychloride</td>
<td>2.112</td>
<td>3.560</td>
<td>1.920</td>
<td>1.824</td>
<td>0.055</td>
</tr>
<tr>
<td>7.5 g/l copper oxychloride</td>
<td>1.555</td>
<td>3.220</td>
<td>1.190</td>
<td>1.856</td>
<td>0.052</td>
</tr>
<tr>
<td>Foliafeed D (0.625 g/l)</td>
<td>1.783</td>
<td>4.350</td>
<td>2.670</td>
<td>1.600</td>
<td>0.056</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>N.S.</td>
<td>1.012</td>
<td>0.552</td>
<td>0.342</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

3-1-2- Effect Of Foliafeed D:

The results indicated that, no significant effect on total chlorophyll concentration was detected by Valencia orange leaves sprayed with the foliafeed D foliar application when compared with control treatment supplied with the recommended rate of micronutrients.

Similar results were obtained by Mohsen et al. (1992) on balady orange trees, El-Shazly et al. (2000) and Gendiah and Hagagy (2000) on Washington navel orange trees, Sourour (2000) on Valencia orange trees and Banuls et al. (2003) on citrus.

In this respect, Bogorad (1966) found that, iron is essential for chlorophyll synthesis; it is necessary for the synthesis of amino levulinic acid a precursor of chlorophyll. Gardner et al. (1985) reported that, significant increases of leaf chlorophyll may be due to the precursor molecules for chlorophyll synthesis includes iron, and if it is not present, chlorophyll can not be synthesized. Spiegel-Roy and Goldschmidt (2008) noticed that, iron is involved in chlorophyll synthesis and is part of certain enzyme systems. It is involved in the reduction-oxidation process in photosynthesis and respiration. Hassan et al. (2010) reported that, iron (Fe) complexes with proteins to form important enzymes in the plant and is associated with chloroplasts, where it has some roles in the synthesizing chlorophyll.

Moreover, Bergmann (1992) reported that, manganese also affects chloroplast formation, chlorophyll synthesis, and therefore photosynthesis and the carbohydrate metabolism. Lack of manganese reduces levels of both green and yellow leaf pigments. This explains why levels of chlorophyll, carotene and photosynthesis increase rapidly when manganese deficiency is corrected by manganese fertilization. Spiegel-Roy and Goldschmidt (1996) reported that, Mn activated several enzyme systems and is required in respiration and photosynthesis and is part of certain enzyme systems. It is involved in the reduction oxidation process in photosynthesis and respiration. Purohit (2007) reported that, manganese plays a direct role in photosynthesis. The sensitivity of chlorophyll towards light destruction increases under conditions of manganese deficiency leads to chlorosis in Chlorella pyrenoidosa. It appears that the site of manganese activity is the oxygen-producing step in photosynthesis. Spiegel-Roy and Goldschmidt (2008) mentioned that, Mn activates several enzyme systems and is required in respiration and photosynthesis.

Mancino et al. (1999) working on bentgrass, found that Mn and Zn are important plant micronutrients involved in chlorophyll synthesis, photosynthesis, various enzyme systems and possibly auxin synthesis. Zude et al. (1999) working on citrus, reported that, iron sufficient plants, the chlorophyll content increased and net photosynthesis rate was enhanced following application of the chelates (7 mg Fe/l). Dawood et al. (2000) working on Washington navel orange trees, found that the highest value of both chlorophyll a and b was obtained with Fe (Fe-EDTA, 50 ppm Fe) treatment followed by (Fe-EDTA, 50 ppm Fe + Mn-EDTA, 50 ppm Mn + Zn-EDTA, 50 ppm Zn) treatment.

3-2- Total Sugars:

3-2-1- Effect Of Copper Oxychloride:

It is clear from the results in Table (4) that, significant and non-significant increases in total sugars concentration were recorded by the leaves of the trees treated with the lowest (0 or 2.5 g/l) or the highest (7.5 g/l) rates of copper oxychloride when compared with those treated with the recommended rate of copper oxychloride (5 g/l).

In this respect, Brown and Clark (1977) and Mizuno et al. (1982) reported that, in plants suffering from copper deficiency the content of soluble carbohydrates is considerably lower than normal during the vegetative stage. Bergmann (1992) reported that, soluble lower carbohydrate concentrations were generally lower in copper-deficient plants only during the initial stages of development and later, when the plants ripening, rose to levels above those of plants with an adequate copper status. Romeu-Moreno and Mas (1999) working on Vitus
vinifera, mentioned that Cu-exposed plants contained lower concentration of soluble sugars than control. Stenko et al. (2009) working on citrus, reported that, copper is necessary for carbohydrate and nitrogen metabolism.

3-2-2- Effect Of Foliafeed D:

The data revealed that, significant increase on total sugars concentration was recorded on leaves of Valencia orange trees treated with foliafeed D foliar application when compared with control plants treated with the recommended rate of micronutrients fertilization. In this respect, Mengel and Kirkby (1979) mentioned that, boron play an essential role in sugar transport and in pentose phosphate pathway. Sun et al. (1987) and Nawar (1992) on Anna apple trees, mentioned that chlorotic leaves of iron deficient plants contained less sugar than green ones. Robson (1993) reported that, the involvement of Zn in carbohydrates metabolism can be demonstrated through its effect on photosynthesis and sugar transformation. Zinc deficiency greatly depressed the activity of aldolase in plant tissue which then impairs the conversion of fructose 1-6-diphosphate to its subsequent compounds. Marschner (1995) reported that, many zinc-dependent enzymes are involved in carbohydrate metabolism in general and of leaves in particular. Besides its function in the carboxic anhydrase reaction, zinc is required for example, for the activity of two other key enzymes, fructose 1-6- bisphosphatase and the aldolase. Rashad and Hanafy Ahmed (1997) working on faba bean, suggested that the increases in sugar concentrations due to Fe and Zn applications (25, 50 and 75 ppm) may be attributed to promote photosynthesis enzymes activity as sucrose synthetase and/or increase chlorophyll structure. Spiegel- Roy and Goldschmidt (2008) working on citrus reported that, boron not readily translocated, appears to be required for sugar translocation. Hassan et al. (2010) mentioned that, manganese (Mn) participates in several important processes including photosynthesis, and metabolism of both nitrogen and carbohydrate.

3-3- Amino Acid and Tryptophan:

3-3-1- Effect Of Copper Oxychloride:

The data revealed that, copper oxychloride has no significant effect on amino acid and tryptophan of Valencia orange leaves sprayed with either the lowest level of copper oxychloride foliar applications (2.5 g/l) or the highest level (7.5 g/l) compared with control treatment(untreated with copper oxychloride) or with trees treated with the middle level of copper oxychloride (5 g/l). significant decrease in total free amino acids concentration was recorded by the leaves of the tree untreated with copper oxychloride (0.0 level), while tryptophan concentration was slightly increase by the some treatments. In this respect, Weber et al. (1991) showed that, copper toxicity inhibits conversion of NH$_4^+$ into amino acids in Silene vulgaris (Moench) Gorcke. Bergmann (1992) reported that, copper is transported through the xylem and phloem as soluble organic copper-nitrogen complex such as copper-amino acids. If copper is deficient, concentration of organic and amino acids, particularly of asparatic acid and asparagines increases.

3-3-2- Effect Of Foliafeed D:

The data in Table (4) revealed that, non-significant increase in tryptophan concentration was detected by the leaves of the trees treated with foliafeed D when compared with control treatment. On the other hand, a reverse trend was obtained by total free amino acids

In this respect, Bergmann (1992) reported that, “genetic iron chlorosis” can lead to amino acids enrichment even if “iron deficiency” actually exists. Marschner (1995) found that, there is no convincing evidence a direct effect of boron on nitrogen metabolism, for example, nitrate reduction, amino acid and protein content, which might be higher or lower in boron deficient plants, depending upon severity of the deficiency, plant age and plant organ. Bisht et al. (2002) working on tomato plants, noticed that plant exposure to Fe deficiency enhanced the accumulation of soluble nitrogenous compounds. Spiegel- Roy and Goldschmidt (2008) working on citrus, mentioned that Mo is involved in the nitrate and nitrate reducing systems acting as an electron carrier.

3-4- Total Soluble Phenols:

3-4-1- Effect Of Copper Oxychloride:

The results indicate that, there is a negative relationship between increasing total soluble phenols concentration and increasing copper oxychloride applications level. The highest values of total soluble phenols
was recorded by the leaves of the trees untreated with copper oxychloride, while the lowest values of total soluble phenols were obtained by the leaves of the trees sprayed with the highest levels of copper oxychloride.

In this respect, it can be suggested that, the effect of copper oxychloride on total soluble phenols concentration may be occur due to the influence of copper element found on copper oxychloride on phenol compounds formation.

In this respect, Judel (1972) working on Chrysanthemum morifolium, reported that under copper deficiency, the decrease in polyphenol oxidase activity is quite severe and is correlated with an accumulation of phenolics and a decrease in the formation of melanotic substances. Marschner (1995) noticed that, copper has a high affinity for peptide and sulfhydryl groups, and thus to cysteine rich proteins, as well as for carboxylic and phenolic groups. Polyphenol oxidase activity in soybean leaves is only lowered by copper deficiency and not by any of the other micronutrient deficiencies. Caldwell (2000) found that, increasing CuSO$_4$.5H$_2$O levels significantly above 100 $\mu$M usually decreased the amount of spinach leaf phenolic compounds. Caldwell (2002) working on spinach plants, reported that CuSO$_4$.5H$_2$O altered the leaves of the phenolic compounds, increasing the levels of some compounds at low Cu (II) concentrations (12.5 ppm) and decreasing the levels of all the phenolic compounds at higher Cu levels (625 ppm). Purohit (2007) reported that, copper acts as a component of phenolases, laccase and ascorbic acid oxidase.

3-4-2- Effect Of Foliafeed D:

The data in Table (4) revealed that, significant and non-significant increases in total soluble phenols concentration were detected by foliafeed D treated trees when compared with control treatment (untreated with copper oxychloride).

In this respect, Engelsma (1972) reported that, Mn is a cofactor for a number of enzymes involved in phenol production; these include phenylalanine ammonialayze. Krishna and Bhorti (1983) working on Avena coleoptile, mentioned that manganese may be involved in hormonal control of phenol synthesis. Brown et al. (1984) working on wheat, suggested that the increase of phenylalanine and tyrosine under high values of manganese might explain the increase in phenol production. Hanafy Ahmed et al. (1996) suggested that, the high values of free amino acids and soluble phenols concentration in broad bean plants which were recorded by using manganese treatment may be attributed to high metabolic activity of plants to synthesize shikimic and chrosomic acids.

3-5- Endogenous Plant Hormones:

3-5-1- Effect Of Copper Oxychloride:

The results in Table (5) revealed that, high values of GA$_3$, IAA and ABA concentrations were recorded by the leaves of Valencia orange trees sprayed with the lowest (2.5 g/l) or the highest (7.5 g/l) rate copper oxychloride foliar applications when compared with control treatment supplied with the recommended rate of copper oxychloride (5 g/l). However, significant decrease in GA$_3$ concentration was recorded by the leaves of Valencia orange trees untreated with copper oxychloride, while a reverse trend on IAA and ABA concentration was obtained by the same treatment.

In this respect, the effect of copper applications on endogenous plant hormones were reported by Brown (1979), Srivastava and Gupta (1996) on crop.

In this connection, Brown (1979) reported that, at high copper supply, certain changes in root morphology such as inhibited elongation and enhanced lateral root formation might be related to the sharp decrease in IAA oxidase activity in roots exposed to high copper concentrations. Srivastava and Gupta (1996) found that, copper-activated phenolases regulate auxin activity in plants. Rashad et al. (2002) working on mung bean (Vigna radiata L.), showed that IAA concentrations were increased relatively to the control with an increase in Cu [copper sulphate 2.5 and 5.0 ppm] rates in kawmi-1. In VC-2719 cultivar, the low rate of Cu (2.5 ppm) gave high value compared with the control.

3-5-2- Effect Of Foliafeed D:

The data in Table (5) indicated that, significant increases in both GA$_3$ and IAA were recorded in the leaves of Valencia orange trees treated with foliafeed D when compared with control treatment. On the other hand, a reverse trend was detected in ABA concentrations.

In this respect, Morgan et al. (1976) found that, manganese influences auxin activity in a complex manner. At both deficient and marginally toxic concentration of Mn, IAA oxidase activity is increased in young leaves of cotton plants, and symptoms of reduced growth and leaf abscission were consistent with those of auxin deficiency in the plants. Burnell (1988), Hughes and WilliaMss (1988) found that, manganese activates several
enzymes of the shikimic acid pathway and subsequent pathways, leading to the biosynthesis of various secondary products, such as IAA.

For boron, Robertson and Loughman (1974) found that boron plays an essential role in the biosynthesis of auxin in the meristem of the plant and boron deficiency leads to a decreased level of free auxin, an increased level of bound auxin and a reduction of IAA-oxidase activity. Boron-deficient plants contained more IAA than control and also had an inhibited IAA-oxidase, presumably because of high levels of phenolic compounds. It is suggested that boron controls the IAA level in plants. It has been also proposed that boron is involved with the metabolism, transport or action of auxin-type hormones. Fuente et al. (1985) found that B has been shown to be essential for IAA transport and has been hypothesized to affect the transport system by both influencing membrane permeability and having some effect on IAA transport protein. Blevins and Lukaszewski (1998) found a close negative correlation between root growth and oxidative degradation of IAA, both greatly affected by boron nutrition. Indole acetic acid oxidation rate was also negatively correlated with root tips ascorbate content, which has been shown to depend on boron nutrition. Hanafy Ahmed et al. (1999) working on peas, reported that IAA was increased in the shoots at all rates of boron foliar applications (0, 100, 250 or 500 ppm) in non-inoculated pea plants under both low and high nitrogen fertilization. Also, the authors concluded that the high activity of free gibberellin in addition to the low activity of ABA might have an effect on the induction of early flowering, protein synthesis and carbohydrates metabolism and ultimately led to an early flower initiation and accumulation leading to the increase of plant growth and seed yield as a result of the foliar application of boron to pea plants.

Moreover, Rashad and Hanafy Ahmed (1997) mentioned that there is hormonal balance between inhibitor and promotive hormones and these ratios may be changed in response to Fe and Zn foliar applications (25, 50 and 75 ppm). All Fe and Zn treatments caused increase of endogenous GA3 in faba bean leaves. It seems to be that iron and zinc might have role in GA3 synthesis, which is related to increase metabolic activity and flowering processes. Hansch and Mendel (2009) reported that iron is importance for life. As redox- active metal it is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone bio-synthesis (ethylene, gibberellic acid, jasmonic acid), production and scavenging of reactive oxygen species, osmoprotection, and pathogen defense.

Table 4: Effect of copper oxychloride and foliafeed D on plant hormones concentrations (GA3 mg/100 g, IAA mg/100 g, ABA mg/100 g, Fe, Mn, Zn and Cu concentrations (mg/g d.w.) of Valencia orange leaves during season 2003.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GA3 (mg/100 g)</th>
<th>IAA (mg/100 g)</th>
<th>ABA (mg/100 g)</th>
<th>Fe (mg/100 g)</th>
<th>Mn (mg/100 g)</th>
<th>Zn (mg/100 g)</th>
<th>Cu (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>460.314</td>
<td>2.632</td>
<td>10.821</td>
<td>186.37</td>
<td>37.87</td>
<td>32.35</td>
<td>12.92</td>
</tr>
<tr>
<td>Cont. zero copper oxychloride</td>
<td>410.012</td>
<td>3.140</td>
<td>13.620</td>
<td>199.59</td>
<td>46.12</td>
<td>35.16</td>
<td>8.82</td>
</tr>
<tr>
<td>2.5 g/l copper oxychloride</td>
<td>480.760</td>
<td>4.160</td>
<td>11.350</td>
<td>205.24</td>
<td>47.90</td>
<td>29.07</td>
<td>16.49</td>
</tr>
<tr>
<td>7.5 g/l copper oxychloride</td>
<td>495.031</td>
<td>3.062</td>
<td>12.480</td>
<td>216.19</td>
<td>41.41</td>
<td>30.51</td>
<td>22.26</td>
</tr>
<tr>
<td>Foliafeed D (0.625 g/l)</td>
<td>575.112</td>
<td>5.730</td>
<td>10.040</td>
<td>291.30</td>
<td>32.97</td>
<td>43.02</td>
<td>14.89</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>32.506</td>
<td>0.439</td>
<td>1.189</td>
<td>59.456</td>
<td>9.927</td>
<td>N.S.</td>
<td>3.487</td>
</tr>
</tbody>
</table>

3-6- Minerals Concentration:

3-6-1- Effect Of Copper Oxychloride:

The data in Table (5) revealed that, Cu and Fe concentrations gradually increased with increasing copper oxychloride foliar application, while no constant trend could be detected on Mn concentrations in leaves with increasing level of copper oxychloride foliar application. However, low values of Mn concentration tended to record by the trees sprayed with the highest level of copper oxychloride (7.5 g/l), with some exceptions. Moreover, the results indicated that, the highest values of Mn concentration in the leaves were obtained by the trees sprayed with the lowest rate of copper oxychloride (2.5 g/l).

Furthermore, the data revealed that, a negative relationship could be detected between increasing copper oxychloride foliar application level (as a source of copper element) and Zn concentration in leaves, with some exceptions. On the other hand, a positive relationship was detected between increasing copper oxychloride foliar application levels and Cu concentration in leaves at the lowest (2.5 g/l) and the recommended (5 g/l) rate of copper oxychloride foliar application levels.

Similar results were obtained by Mann (1980) on sweet orange, Bergmann (1992), Mozaffari et al. (1996) on citrus rootstocks and Alva et al. (1999) on citrus seedlings.

In this respect, Bergmann (1992) found that, zinc uptake can be inhibited by high iron and copper contents of plants whereas manganese has no appreciable effect. It is believed that, Fe3+, Fe2+ and Cu2+ ions can displace zinc more easily than Mn2+ owing to their better chelations properties. Alva and Chen (1995) found a decrease
in uptake of Zn seedlings of Cleopatra mandarin and Swingle citrumelo rootstock with an increase in Cu concentrations from 0.1 to 20 μM in nutrient solution at pH 5.5. Vasconcelos et al. (1997) evaluated the phytoavailability of copper in three doses (0.0, 2.5 and 5.0 mg/dm³ soil) in soybean. Huang and Alva (1999) working on Swingle citrumelo seedlings, found that the Cu concentration in the leaves increased substantially with an increase in the Cu rates (0, 25, 50, 100, 200 and 400 mg/kg) applied to the candler and Myakka fine sands, while the effect was marginal in the oldsmar fine sand. Also, the authors added that, the total content of Zn and Fe in the leaves, significantly decreased with increase in Cu rates. Rashad et al. (2002) working on mung bean, reported that the low rate of Cu foliar application (2.5 ppm) caused highly increase Fe concentration in leaves and roots when compared with control-untreated plant.

3-6-2- Effect Of Foliafeed D:

The results revealed that, significant and non-significant increases in Fe, Zn and Cu concentrations were detected in leaves of the trees supplied with foliafeed D foliar application when compared with control treatment. On the other hand, an opposite trend was obtained by Mn concentration in leaves.

In this respect, Carpena et al. (1976) found that, increment of Fe concentration may be attributed to the involvement of most spraying Fe in metabolic processes and contribution in the formation of organic iron compound. Shawky et al. (1993) working on Washington navel orange trees, found that the content of Zn and Mn in leaves was slightly increased by increasing the concentration of solution (each of Zn, Mn, Cu and Fe were applied at 0.0, 1.25, 2.50 and 3.75 ppm whereas Ca and Mg were at rate of 0.0, 2.50, 5.0 and 7.50 ppm in aforementioed rates, respectively). El-Shazly et al. (2000) working on Washington navel orange, showed that both leaf trees Fe soluble and assimilated fractions significantly increased as a result of Fe foliar spray in both seasons. Banuls et al. (2003) working on citrus, found that the soil Fe-EDDHA application (3 g Fe/tree) affected significantly the concentrations of micronutrients: Fe and Mn concentrations increased in comparison to control ones. El-Baz (2003) working on balady mandarin trees, found that the different applied zinc and boron sprays (Zn sulphate solution at 250 or 500 ppm Zn, boric acid solution at 40 or 80 ppm B, Zn at 250 ppm + B 40 ppm or Zn at 500 ppm + B 80 ppm) increased N, K, Mn, Zn and B contents above the incipient deficiency level to the optimum level compared with the unsprayed trees.

2- Yield And Physical Characters Of Valencia Orange Fruits:

2-1- Effect Of Copper Oxychloride:

The data in the Table (3) indicated that, in the two successive seasons, significant increases in tree yield (kg per tree), single fruit weight (g) and fruit size (cm³) were recorded by the fruits of Valencia orange trees sprayed with either the lowest level of copper oxychloride foliar applications (2.5 g/l) or the highest level (7.5 g/l) compared with control treatment in which trees were treated with the middle level of copper oxychloride (5 g/l) or trees untreated with copper oxychloride. However, no effect could be trend was detected on number of fruits per tree in the two successive seasons.

These results are in agreement with those obtained by Sharma et al. (1999) on seedless lemon and Ram and Bose (2000) on mandarin trees.

In this respect, Reuter et al. (1981) reported that, a decline in polyphenol oxidase activity with copper deficiency may be at least indirectly responsible for the delay in flowering and maturation often observed in the copper-deficient chrysanthemum. Copper deficiency led to a decrease in the number of flowering shoots, but mainly prevented the opening of flowers. Marschner (1995) reported that, the Cu enzymes phenolase and laccase are very important for lignification. These enzymes oxidase p-coumaric acid, precursor of lignin biosynthesis. This role in lignification may be why Cu is more important for seed and fruit production than for vegetative growth. The main reason for the reproductive growth is nonviability of pollen from Cu-deficient wheat plants (Graham, 1975). Brown (1990) found that, increased firmness in copper-treated apricots raises questions about the physiological effects of foliar applied copper on developing fruit. Bergmann (1992) reported that, in potato, tobacco and vine plants, for instances physiological ageing can be delayed by spraying with copper salt solutions, thus extending the growth period and improving yields. Baker and Senft (1995) reported that, insufficient Cu-supply to plants can lead to subclinical or hidden deficiency, which may be as severe to cause yield losses of around 20% with no symptoms can be obviously recognized.

2-2- Effect Of Foliafeed D:

The results revealed that, significant and non- significant increases in tree yield, single fruit weight (g) as well as fruit size (cm³) in the second season were detected by foliafeed D treated trees when compared with control treatment. However, no constant trend could be recorded on number of fruits per trees in the two seasons.

Table 5: Effect of copper oxychloride and foliafeed D on yield and fruit quality of Valencia orange trees during (2003 and 2004) seasons.

<table>
<thead>
<tr>
<th>Season</th>
<th>Treatments</th>
<th>Tree yield (kg)</th>
<th>Number of fruits per tree</th>
<th>Single Fruit weight (g)</th>
<th>Fruit size (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Cont.</td>
<td>34.84</td>
<td>290</td>
<td>120.12</td>
<td>122.33</td>
</tr>
<tr>
<td></td>
<td>Cont. zero copper oxychloride</td>
<td>33.12</td>
<td>295</td>
<td>112.33</td>
<td>123.13</td>
</tr>
<tr>
<td></td>
<td>2.5 g/l copper oxychloride</td>
<td>44.17</td>
<td>293</td>
<td>150.70</td>
<td>148.16</td>
</tr>
<tr>
<td></td>
<td>7.5 g/l copper oxychloride</td>
<td>47.85</td>
<td>290</td>
<td>165.02</td>
<td>167.10</td>
</tr>
<tr>
<td></td>
<td>foliafeed D</td>
<td>35.61</td>
<td>295</td>
<td>120.87</td>
<td>118.33</td>
</tr>
<tr>
<td></td>
<td>L.S.D. (0.05)</td>
<td>3.54</td>
<td>N.S.</td>
<td>8.82</td>
<td>8.29</td>
</tr>
<tr>
<td>2004</td>
<td>Cont.</td>
<td>38.22</td>
<td>310.0</td>
<td>123.30</td>
<td>117.73</td>
</tr>
<tr>
<td></td>
<td>Cont. zero copper oxychloride</td>
<td>30.92</td>
<td>280.3</td>
<td>110.31</td>
<td>127.31</td>
</tr>
<tr>
<td></td>
<td>2.5 g/l copper oxychloride</td>
<td>46.09</td>
<td>303.33</td>
<td>151.90</td>
<td>155.65</td>
</tr>
<tr>
<td></td>
<td>7.5 g/l copper oxychloride</td>
<td>46.76</td>
<td>276.00</td>
<td>169.38</td>
<td>164.38</td>
</tr>
<tr>
<td></td>
<td>foliafeed D</td>
<td>45.34</td>
<td>290.00</td>
<td>156.31</td>
<td>150.31</td>
</tr>
<tr>
<td></td>
<td>L.S.D. (0.05)</td>
<td>4.37</td>
<td>23.65</td>
<td>8.30</td>
<td>7.30</td>
</tr>
</tbody>
</table>

In this respect, Nijjar (1985) working on Balady mandarin trees, reported that effect of both zinc and boron application on size and weight could be attributed to their involvement in cell division and cell elongation. Generally, the increase in the available nutrients for mandarin trees after spraying both Zn and B or their combinations leads to improve the formation of cell wall components such as cellulose, lignin and preventing formation of abscission layer, consequently, reduction of preharvest abscission layer formation, consequently, reduction of preharvest fruit dropping. Miller et al. (1994) found that, Fe chelates [Libfer (EDDHA), Micrel 650 (EDDHMA)] are applied in spring (25 August – 15 September) as spot treatments to Citrus trees showing 40% or greater chlorosis; the cost would be offset by the increase in yield/tree and the additional expense of applying FeSO₄ would be justified by improved fruit size. Dai et al. (1995) working on Pongan mandarin, reported that boron, zinc, molybdenum and calcium had positive effects on flower bud formation. Patel et al. (1997) working on acid lime trees, stated that 0.5% FeSO₄ with 0.05% citric acid application could effectively control chlorosis as well as fruit yield. Fruit yield was closely associated with the decrease in chlorosis. Sarawathi et al. (1998) reported that, the yield of mandarin orange (Citrus reticulata Blanco), number of fruits/plant and fruit weight were improved by Zn + Mn. Dawood et al. (2000) working on Washington navel orange trees, found that the highest values of fruit set and fruiting % were obtained with (Zn 50 ppm + Mn 50 ppm) treatment which gave the lowest values of June drop and preharvest fruit drop %. This may be due to the enhancement of tree nutrition status and plant growth. El-Shazly et al. (2000) mentioned that, the increment of Washington navel orange yield as a result of Fe-EDDHA sprays may be attributed to its function in enzymatic systems and chlorophyll formation and consequently increased photosynthesis and saved a sufficient amount of carbohydrates and other assimilates which finally increased the yield. Ram and Bose (2000) reported that, the vigorous growth and better source sink relationship may be the reason for enhanced fruit yield on Karma Khatta rootstock, also reported increased fruit yield by the use of Mg, Cu, Zn, Fe and B alone or various combinations in mandarin. Banuls et al. (2003) working on citrus, pointed out that fruit yield significantly increased of about 18% in the Fe-treated trees when compared to control-untreated trees, and this was due to higher number of fruits by trees treated with Fe-EDDHA. Mishra et al. (2003) found that, the better response of micronutrients treatments over control particularly, Zn [ZnSO₄ (0.5%) + B (Boric acid 0.2%) and Fe (FeSO₄ 0.4%) + B (Boric acid 0.2%)] treatments of fruit yield was probably due to increase in fruit weight of Kinnow mandarin (on different rootstocks) and juice percentage by micronutrient application.

References


