Effect of foliar application of micronutrients and soil application of zinc sulphate on total chlorophyll and carotenoid content in sweet orange (*Citrus sinensis*) cv. blood red

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Abstract

The present investigation was carried out for two consecutive years of 2016 and 2017 at experimental orchard of Department of Horticulture, CCS HAU, Hisar to assess the performance of sweet orange cv. Blood Red to foliar application of micronutrients and soil application of zinc sulphate. In experiment I, the foliar application of micronutrients was done two times i.e. Ist spray was done on first week of April and 2nd spray on first week of July. The experiment comprises of thirteen treatments, viz., Zinc sulphate (ZnSO$_4$) @ 0.25%, 0.50% &1.00%; Iron sulphate (FeSO$_4$) @ 1.5%, 2.0% & 2.5%; Manganese sulphate (MnSO$_4$) @ 0.25%, 0.50% & 1.00%; Boric acid (HBO$_3$) @ 0.1%, 0.2% & 0.3% and Control (No micronutrients). In experiment II, the soil application of zinc sulphate (ZnSO$_4$) was done two times i.e. half dose in second fortnight of February and half dose in second fortnight of May. The experiment comprises of six treatments, viz., Zinc sulphate (ZnSO$_4$) @ 50 g/plant, 100 g/plant, 150 g/plant, 200 g/plant, 250 g/plant and Control (No Zn application). Results indicated that foliar application of micronutrients and soil application of zinc sulphate affected significantly the chlorophyll and carotenoid content.

Keywords: *Citrus sinensis*, foliar application, soil application, micronutrients, sweet orange

Introduction

The genus *Citrus* is economically very important and is known for its juice and pulp throughout the world. Citrus is one of the choicest fruit with high consumer preference both as fresh fruit as well as for its refreshing processed juice. It is extensively grown in tropical and sub-tropical regions. Citrus is a commercially important fruit crop of India and grown across its length and breadth with a production of 13200 thousand MT from an area of 1034 thousand hectares during year of 2018-19 (Anonymous, 2018-19) [1]. Sweet orange (*Citrus sinensis* (L.) Osbeck) has been reported to be originated in Southern China and it was introduced to India during thirteenth century (Swingle, 1943; Webber, 1948) [9, 11]. It is the second largest citrus fruit, cultivated in tropical and subtropical regions of the country. Total sweet orange production in India is 3401 thousand MT with an area of 190 thousand hectares during year of 2018-19 (Anonymous, 2018-19) [10]. The productivity of sweet orange mainly depends on adequate supply of plant nutrients seems to be a very important factor in regulating cropping and influencing the quality of sweet orange. The micronutrients are required in small amounts, but play a great role in plant metabolism (Kazi et al., 2012) [4]. Despite some shortcomings, foliar application is regarded as the best method under certain conditions (Marschner and Marschner, 2012) [8]. Foliar or soil application of Zn increases the biosynthesis of chlorophyll and carotenoid synthesis that are important for proper performance of photosynthetic process (Mousavi, 2011) [7]. The beneficial effects of amelioration of zinc, iron, manganese and boron deficiencies by foliar application have been documented by previous studies (Hippler et al., 2015) [3]. The present investigation has been undertaken to study the effect of foliar application of micronutrients and soil application of zinc sulphate on chlorophyll and carotenoid content of fruit sweet orange.

Material and Methods

The present investigation was carried out on fifteen years old sweet orange cv. Blood Red trees planted at a spacing of 6m X 6m in experimental orchard of Department of Horticulture, CCS HAU, Hisar, Haryana, India.
HAU, Hisar situated at 215.2 m above mean sea level with coordinates of 29º10ʹ N latitude and 75º46ʹ E longitudes, during the year 2016-17 and 2017-18. In Experiment I, the foliar application of micronutrients was done two times i.e. Ist spray was done on first week of april and 2nd spray on first week of july. The experiment comprises of thirteen treatments, viz., Zinc sulphate (ZnSO₄) @ 0.25%, 0.50% &1.00%; Iron sulphate (FeSO₄) @ 1.5%, 2.0% & 2.5%; Manganese sulphate (MnSO₄) @ 0.25%, 0.50% & 1.00%; Boric acid (H₃BO₃) @ 0.1%, 0.2% & 0.3% and Control (No micronutrients). In experiment II, the soil application of zinc sulphate (ZnSO₄) was done two times i.e. half dose in second fortnight of february and half dose in second fortnight of May. The experiment comprises of six treatments, viz., Zinc sulphate (ZnSO₄) @ 50 g/plant, 100 g/plant, 150 g/plant, 200 g/plant and Control (No Zn application). Treatments were allocated in randomized block design (RBD) with three replications in each treatment. The recommended standard package of practices and plant protection measures were adopted to keep the plants in good health. Methods used to measure the chlorophyll and carotenoid content are as follows:-

**Total chlorophyll and carotenoid Content:** Chlorophyll pigments namely chl. a, chl. b, total chlorophyll and carotenoids were estimated in the fruits taken from different sampling stages by the method given by Wellburn (1994) [12] and expressed in terms of µ moles/g fresh weight of peel tissue.

| Treatments | Total Chlorophyll content 2016 | Total Chlorophyll content 2017 |
|------------|-------------------------------|-------------------------------|
| ZnSO₄ 0.25% | 180.86                        | 182.10                        |
| ZnSO₄ 0.50% | 182.03                        | 183.71                        |
| ZnSO₄ 1.00% | 183.92                        | 185.22                        |
| FeSO₄ 1.5%  | 185.44                        | 186.89                        |
| FeSO₄ 2.00% | 187.24                        | 188.14                        |
| FeSO₄ 2.5%  | 189.91                        | 189.80                        |
| MnSO₄ 0.25% | 176.35                        | 177.78                        |
| MnSO₄ 0.50% | 177.78                        | 179.12                        |
| MnSO₄ 1.00% | 179.21                        | 180.36                        |
| H₂BO₃ 0.1%  | 172.36                        | 174.18                        |
| H₂BO₃ 0.2%  | 173.39                        | 175.01                        |
| H₂BO₃ 0.3%  | 174.98                        | 176.37                        |
| Control     | 171.12                        | 172.81                        |
| C.D. at 5%  | 1.16                          | 1.12                          |

The data concerning total chlorophyll content is presented in Table 1, which indicates that different concentrations of ZnSO₄, FeSO₄, MnSO₄ and H₂BO₃ affected total chlorophyll content significantly during both the years. In year 2016, maximum (189.91) total chlorophyll content was observed with FeSO₄ 2.5% followed by FeSO₄ 2.00% (187.24) and minimum (171.12) in control. Similar trend was observed in the year 2017, showing maximum (189.80) total chlorophyll content was observed with FeSO₄ 2.5% followed by FeSO₄ 2.00% (188.14) and minimum (172.81) in control, respectively. The results on total chlorophyll were found significant with foliar application of micronutrients. The highest total chlorophyll was observed with FeSO₄ 2.5%. An increase in total chlorophyll content with foliar application of micronutrients might be due to delayed chlorophyll degradation. The results are in conformation with Shahin et al. (2010) [18] in apple and Eiada and Al-Hadethi (2013) [2] in pomegranate. This might also be due to delayed senescent pigment changes and oppose the ethylene induced loss of chlorophyll (Trebbitsh et al., 1993) [10].

**Extraction of pigments:** Two discs (0.28 cm² area) of the fruit peel were suspended in a test tube containing 10 ml of dimethyl sulfoxide (DMSO). The weight of the discs was recorded before putting them into test tubes. The test tubes were then placed in an oven at 60°C for four hours to facilitate the extraction of pigments.

**Estimation:** After the incubation period for extraction, the test tubes were cooled to room temperature and the optical density was read at 454, 645 and 665 nm on a spectrophotometer (Spectronic-20D). DMSO was used as blank. The chlorophyll pigments were calculated by using the following equations:-

\[
\text{Chlorophyll - a (µg/ml)} = 12.19 \times A_{665} - 3.45 \times A_{645} \\
\text{Chlorophyll - b (µg/ml)} = 21.99 \times A_{665} - 5.32 \times A_{645} \\
\text{Total Chlorophyll (µg/ml)} = \text{Chlorophyll a + Chlorophyll b}
\]

\[
\text{Carotenoids (µg/ml)} = \frac{(1000 \times A_{454} - 2.86 \times \text{chl. a} - 129.2 \times \text{chl. b})}{221}
\]

The quantity of the above pigments were calculated in mg g⁻¹ tissue fresh weight and expressed in µ moles/g tissue fresh weight by using the following relationship:-

\[
\mu \text{ moles of Chlorophyll – a} = \frac{µ \text{ g chl. a} \times X}{1.119} \\
\mu \text{ moles of Chlorophyll – b} = \frac{µ \text{ g chl. b} \times X}{1.102} \\
\mu \text{ moles of Carotenoids} = \frac{µ \text{ g carotenoids} \times X}{1.809}
\]

**Results and Discussion**

The results are in conformation with Shahin et al. (1990) [20] in apple and Eiada and Al-Hadethi (2013) [2] in pomegranate. This might also be due to delayed senescent pigment changes and oppose the ethylene induced loss of chlorophyll (Trebbitsh et al., 1993) [10].
Table 2: Effect of foliar application of micronutrients on carotenoid content (µ moles/g fr. wt.) of sweet orange cv. Blood Red

| Treatments | Carotenoid content 2016 | Carotenoid content 2017 |
|------------|-------------------------|-------------------------|
| ZnSO₄ 0.25% | 246.55                  | 249.67                  |
| ZnSO₄ 0.50% | 243.01                  | 247.09                  |
| ZnSO₄ 1.00% | 242.89                  | 245.80                  |
| FeSO₄ 1.5% | 241.08                  | 243.19                  |
| FeSO₄ 2.00% | 238.89                  | 241.98                  |
| FeSO₄ 2.5% | 235.78                  | 238.87                  |
| MnSO₄ 0.25% | 251.17                  | 253.19                  |
| MnSO₄ 0.50% | 249.61                  | 251.65                  |
| MnSO₄ 1.00% | 248.02                  | 250.12                  |
| H₂BO₃ 0.1% | 255.50                  | 257.45                  |
| H₂BO₃ 0.2% | 254.26                  | 256.56                  |
| H₂BO₃ 0.3% | 252.60                  | 254.62                  |
| Control     | 257.01                  | 259.01                  |
| C.D. at 5%  | 1.41                    | 1.20                    |

The data presented in Table 2 indicate that different concentrations of ZnSO₄, FeSO₄, MnSO₄ and H₂BO₃ significantly affected carotenoid content of sweet orange cv. Blood Red fruits during both the years. In the year 2016, the highest carotenoid content (257.01) was found in control followed by H₂BO₃ 0.1% (255.50) and lowest (235.78) in FeSO₄ 2.5%. During the year 2017, highest carotenoid content (259.01) was found in control followed by H₂BO₃ 0.1% (257.45) and lowest (238.87) in FeSO₄ 2.5%. The results on content were found significant with foliar application of micronutrients. The highest carotenoid content was found in control whereas lowest in FeSO₄ 2.5%. A decrease in carotenoid content with foliar application of micronutrients might be due to inhibition of carotenoid beta-cryptoxanthin biosynthesis and its accumulation, which inhibit the development of citrus fruit colour and luster. The results are in conformation with Shahin et al. (2010) [8] in apple and Eiada and Al-Hadethi (2013) [2] in pomegranate.

Table 3: Effect of soil application of zinc sulphate on total chlorophyll content (µ moles/g fr. wt.) of sweet orange cv. Blood Red

| Treatments | Total chlorophyll content 2016 | Total chlorophyll content 2017 |
|------------|-------------------------------|-------------------------------|
| ZnSO₄ 50 g/plant | 176.44                        | 176.92                        |
| ZnSO₄ 100 g/plant | 177.98                        | 178.02                        |
| ZnSO₄ 150 g/plant | 179.76                        | 180.23                        |
| ZnSO₄ 200 g/plant | 181.48                        | 182.91                        |
| ZnSO₄ 250 g/plant | 184.67                        | 185.01                        |
| Control     | 172.78                        | 173.66                        |
| C.D. at 5%  | 1.54                          | 1.24                          |

Data presented in Table 3 revealed that total chlorophyll content was significantly affected by various ZnSO₄ concentrations during both the years. In year 2016, the maximum total chlorophyll content (184.67) was observed with ZnSO₄ 250g/plant followed by ZnSO₄ 200g/plant (181.48) and minimum (172.78) in control. In year 2017, the maximum total chlorophyll content (185.01) was observed with ZnSO₄ 250g/plant followed by ZnSO₄ 200g/plant (182.91) and minimum (173.66) in control. The highest total chlorophyll was observed with ZnSO₄ 250g/plant. This might be because zinc delayed chlorophyll degradation. Zinc application enhances the rate of photochemical reductions and chlorophyll content in cucumber (Kazemi, 2013) [5]. The soil application of Zn increases the biosynthesis of chlorophyll that is important for proper performance of photosynthetic process (Mousavi, 2011) [7].

Table 4: Effect of soil application of zinc sulphate on carotenoid content (µ moles/g fr. wt.) of sweet orange cv. Blood Red

| Treatments  | Carotenoid content 2016 | Carotenoid content 2017 |
|-------------|-------------------------|-------------------------|
| ZnSO₄ 50 g/plant | 252.14                  | 254.23                  |
| ZnSO₄ 100 g/plant | 250.54                  | 252.14                  |
| ZnSO₄ 150 g/plant | 248.89                  | 250.53                  |
| ZnSO₄ 200 g/plant | 245.12                  | 247.91                  |
| ZnSO₄ 250 g/plant | 243.33                  | 245.03                  |
| Control     | 254.48                  | 256.11                  |
| C.D. at 5%  | 1.12                    | 1.78                    |

Data given in Table 4 indicated that carotenoid content was significantly affected by various ZnSO₄ concentrations during both the years. In year 2016, the carotenoid content was found maximum (254.48) in control followed by ZnSO₄ 50g/plant (252.14) and minimum (243.33) with ZnSO₄ 250g/plant. In the next year 2017, the carotenoid content was recorded maximum (256.11) in control followed by ZnSO₄ 50g/plant (254.23) and minimum (245.03) with ZnSO₄ 250g/plant . The results carotenoid content were found significant with soil application of zinc sulphate. The highest carotenoid content was found in control. An decrease in carotenoid content might be because zinc inhibit carotenoid beta-cryptoxanthin biosynthesis and its accumulation, which inhibit the development of citrus fruit colour and luster. The results are in conformity with Shahin et al. (2010) [8] in apple and Eiada et al. (2013) [2] in pomegranate.

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