ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.31071-31076, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

BIOMETRIC CHARACTERISTICS AND VISUAL DIAGNOSIS OF SAFFLOWER PLANTS UNDER MACRO AND MICRONUTRIENT OMISSION

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ARTICLE INFO

Article History:
Received 17th February, 2016
Received in revised form
24th March, 2016
Accepted 21st April, 2016
Published online 20th May, 2016

Key words:

Carthamus tinctorius L.,
Plant mineral nutrition,
Deficiency symptoms,
Leonard vase.

ABSTRACT

Safflower is primarily intended to produce oil for human consumption, having the potential for biodiesel production. This study aimed to evaluate macro and micronutrients omission effect on biometrics and visual diagnosis of nutritional deficiencies in safflower plants grown in nutrient solution. Research was conducted in a greenhouse. Nutrient solutions containing all nutrients and with individual omissions of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, molybdenum and zinc were used. The design was completely randomized, with four repetitions. Visual diagnosis of nutrient deficiency symptoms and biometric characteristics were assessed. Data were subjected to analysis of variance and Scott-Knott's test was applied when significant, both up to 5% probability. Among primary macronutrients, nitrogen, followed by phosphorus, caused the highest reductions in all the analyzed variables in safflower plants. Among secondary macronutrients, calcium absence caused the highest reductions, and the need for these three nutrients did not allow for plants to complete their life cycle. Macronutrient omission showed the most severe symptoms in relation to micronutrients.

Citation: Edna Maria Bonfim-Silva, Adriano Bicioni Pacheco, Tonny José Araújo da Silva, Éllen Souza do Espirito Santo, Carina Sthefanie Lemes e Lima Bär and Helon Hébano de Freitas Sousa, 2016. "Biometric characteristics and visual diagnosis of safflower plants under macro and micronutrient omission", *International Journal of Current Research*, 8, (05), 31071-31076.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a herbaceous plant that has good adaptability to hot and dry weather due to deep root system. Its production is mainly intended for grains to produce vegetable oil, which has high quality for human consumption and has potential for biodiesel production (Dordas and Sioulas, 2008, Anicésio *et al*., 2015; Bonfim-Silva *et al*., 2015). Safflower is an important alternative in crop rotation, especially in Brazil. However, there are few basic studies in the plant nutrition area, such as studies about symptomatology by nutrient omission. As plant development is directly related to accurate fertilization and soil correction, it is believed that these studies contribute to increase yield, promoting better efficiency in the use of inputs and improving safflower crop expansion.

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Visual diagnosis of nutrient deficiency symptoms in plants is an important parameter in crop management decision-making. Diagnosis is obtained by nutrient subtraction studies, using nutrient solutions to omit the chemical element that will identify the symptom (Taiz and Zeiger, 2013). Martin *et al*. (1971) observed nutritional deficiency symptoms in safflower crops in field and identified phosphorus deficiency as the consequence through nutrient omission in a nutrient solution. Thus, the importance of visual diagnosis studies on safflower crop nutritional management is highlighted. Given the above, this study aimed to evaluate the effect of macro and micronutrient omission on biometric characteristics and to evaluate the visual diagnosis of nutritional deficiency symptoms of safflower plants grown in nutrient solution.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse in the Institute of Agricultural Sciences and Technology, Federal University of Mato Grosso, Rondonópolis, MT, Brazil, located at latitude

16º27'54.96"S, longitude 54º34'41.79"W and altitude of 229 meters. Experiment conduction took place in the period from February to April 2014.The experimental design was completely randomized, with treatments established in relation to the nutrient solution. Treatments were made with all nutrients (full) and with individual omission of the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn), for a total 13 treatments with four repetitions , totaling 52 experimental units.Vases were used as experimental unit based on the Leonard principle (Vincent, 1970) by adapting PET bottles (Polyethylene Terephthalate) of two liters, which were cut with sufficient height to fill the upper parts with 1 dm^3 sand. Holes with 1 cm diameter were made in the lids to put a wool wick. The bottom of the bottle was used as nutrient solution reservoir. Painting with aluminum was carried out to prevent algae proliferation. Adhesive tape was glued on the vase side to mark the solution level and observe the consumption (Bonfim-Silva *et al*., 2011; Porto *et al*., 2013; Bonfim-Silva *et al*., 2015). A wick was made with 14 woollen yarns having 25 cm long, which was placed through the lid hole to establish contact with the sand, in order to provide the nutrient solution by capillarity. Reservoir reposition was carried out to a volume of about 400 mL nutrient solutions (Porto *et al*., 2013; Bonfim-Silva *et al*., 2015).

Stock and nutrient solutions were prepared with deionized water, as described by Hoagland and Arnon (1950). Nutrient solutions were prepared from stock solutions according with treatment. Nutrient solution pH was adjusted to 5.5 ± 0.5 using a digital pH meter and HCl or NaOH solutions, both at 1.0 N. Nutrient solutions were stored in a refrigerator at 4 °C. Initially, deionized water was provided up to seeding, previously saturating sand by wool wick. From this step, water was replaced by nutrient solution, and the respective treatments were applied. Five seeds were sowed by vase of the IMA 0213 cultivar safflower at a depth of about 0.02m. Thinning was carried out when plants showed the first pair of true leaves, which occurred at eight days after emergence, and two plants were kept per vase.

The vase reservoir was refilled with nutrient solution on a daily basis, according to consumption, and complete exchange of the nutrient solution from the reservoir was conducted every 15 days, in order to keep nutrient levels in balance. Plant height, number of leaves and capitulum, stem diameter at 0.01 m from the plant collar and shoot, root and capitulum dry matter were assessed in the capitulum formation stage, 34 days after emergence. Plants were cut next to the sand, and shoots and roots were separated. Roots were separated from the sand by washing in running water and sieving with a 2 mm mesh sieve. Roots were also separated from the wool wick. Matter drying was conducted in a forced circulation air oven at 65 °C, until constant matter was obtained. Subsequently, weighing was conducted in a semi-analytical balance. Variables were subjected to analysis of variance by F test until 5% probability, and when significant, Scott-Knott test was applied using the SISVAR statistical program (Ferreira, 2008). Nutrient deficiency visual symptoms were described and photographed in order to conduct visual diagnosis study.

RESULTS AND DISCUSSION

There was significant difference up to 5% probability by the F test for all variables. Among primary nutrient omissions, nitrogen and phosphorus omission were grouped as those with the highest negative influence over analyzed variables. Among secondary macronutrients, calcium absence caused less development for safflower plants (Figure 1; Table 1). Safflower plants with nitrogen deficiency had little development since the initial stage, influencing all variables. In the evaluation, plants did not complete the cycle (Table 1). Dordas and Sioulas (2008), Kulekci *et al*. (2009), Haghighati (2010), Taleshi *et al*. (2012), Anicésio *et al*. (2015) and Bonfim-Silva *et al*. (2015) also observed reduction of safflower productive characteristics with nitrogen fertilization absence. Deficiency visual symptoms in safflower leaves began with vein chlorosis, which became widespread throughout the leaf (Figure 2), progressing to necrosis of older leaves, in accordance to what was described by Kumar and Sharma (2013) for safflower plants. According to Taiz and Zeiger (2013), those are common symptoms in crops, as nitrogen is a basic constituent of proteins, enzymes, chlorophyll and nucleic acids in plants, participating of hormone synthesis, which explains the little development of safflower plants.

Phosphorus absence reduced plant development since the initial growth phase, differing from the complete treatment in all the variables analyzed, producing no capitulum (Table 1). Abbadi and Gerendás (2011) and Abbadi and Gerendás (2012) studied phosphate fertilization and observed safflower production increase with phosphorus availability at adequate levels. Symptomlogy caused by phosphorus omission were necrotic spots on the leaf edges, followed by widespread leaf chlorosis (Figure 2) until total necrosis of older leaves. Phosphorus deficiency symptoms corroborated with Martin *et al*. (1971), who reported deficiency symptoms in safflower plants grown in rotation with rice (*Oryza sativa* L.). In order to identify which nutrient caused deficiency, nutrient omission in the nutrient solution was studied and phosphorus deficiency was identified. Therefore, the importance of visual diagnosis studies in a greenhouse with field application is emphasized. According to Taiz and Zeiger (2013), phosphorus in plants is important for energy metabolism. This element is found in the plasma membrane constitution and has good mobility, justifying the low development of safflower plants in this study. Potassium omission in safflower plants caused lower plant growth, negatively influencing all variables. However, it differed from the other primary macronutrients by producing capitulum (Table 1). Abbadi *et al*. (2008) and Vafaie *et al*. (2013) studied potassium fertilization levels in safflower crops and observed that fertilization absence impaired all variables, including grain and oil production. Symptoms caused by potassium deficiency are initially expressed by chlorosis in the leaf border in the shape of an inverted "V", evolving to the leaf center and causing necrosis of older leaves (Figure 2). According to Taiz and Zeiger (2013) and Kumar and Sharma (2013), these are classic potassium deficiency symptoms in plants. According to Taiz and Zeiger (2013), potassium is one of the main enzyme activators. In addition, it is responsible for assimilate translocation and regulation of osmotic pressure in

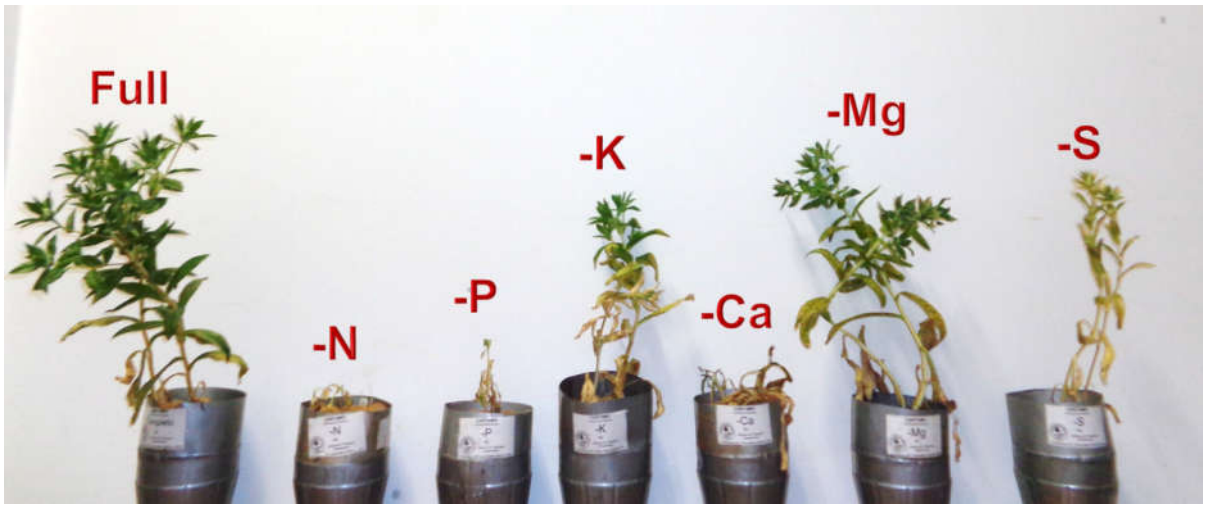

Figure 1. Comparative between safflower plants in relation to complete treatment and macronutrient omission

Table 1. Safflower plant biometric indices in relation to complete treatment and macro and micronutrient omission

Treatments	Plant height (cm)	Leaves (nº pot^{-1})	Stem diameter (mm)	Capitulum (nº pot^{-1})	Capitulum dry mass (g pot^{-1})	Dry mass of shoot (g pot^{-1})	Dry mass of root (g pot^{-1})
Complete	42,3 a	83,0 a	5,18 a	11,0 a	2,1 a	7,9 a	1,63 a
- N	3,2 c	3,0 b	1,49 c	0,0 d	0,0 d	0,1 c	0,05 c
- P	9,0 b	5,0 b	1,59 c	0,0 d	0,0 d	0,3 c	0,12 c
- K	26,1 b	23,5 b	3,77 b	3,2 c	0,4 d	1,9 c	0,39 c
- Ca	11,2 b	9,5 b	3,86 b	0,0 d	0,0 d	1,2 c	0,31 c
- Mg	33,6 b	56,7 a	5,05 a	8,5 b	1,3 b	6,1 b	1,20 a
- S	29,1 b	23,2 b	2,43 c	2,2 c	0,3 d	1,5 c	0,42 c
- B	38,5 a	68,0 a	4,29 b	5,0 c	0,5 d	5,8 b	0,76 b
- Cu	38,9 a	76,0 a	5,07 a	8,7 b	1,7 b	7,6 a	1,28 a
- Fe	40,9 a	73,5 a	5,40 a	11,7 a	2,1 a	9,3 a	1,34 a
- Mn	35,7 a	60,5 a	5,06 a	6,5 c	1,0 c	5,9 b	0,70 b
- Mo	41,2 a	79,0 a	5,07 a	10,2 a	2,3 a	9,0 a	1,43 a
- Zn	39,9 a	69,5 a	4,76 a	8,7 b	1,6 b	7,3 a	1,28 a
Sign. (%)	*0,1*	*0,1*	*0,1*	*0,1*	*0,1*	*0,1*	*0,1*
CV (%)	*13,84*	*29,94*	*14,03*	*34,7*	*35,06*	*24,56*	*33,34*

Means followed by the same letter in the column do not differ by Scott-Knott's test at 5% probability.

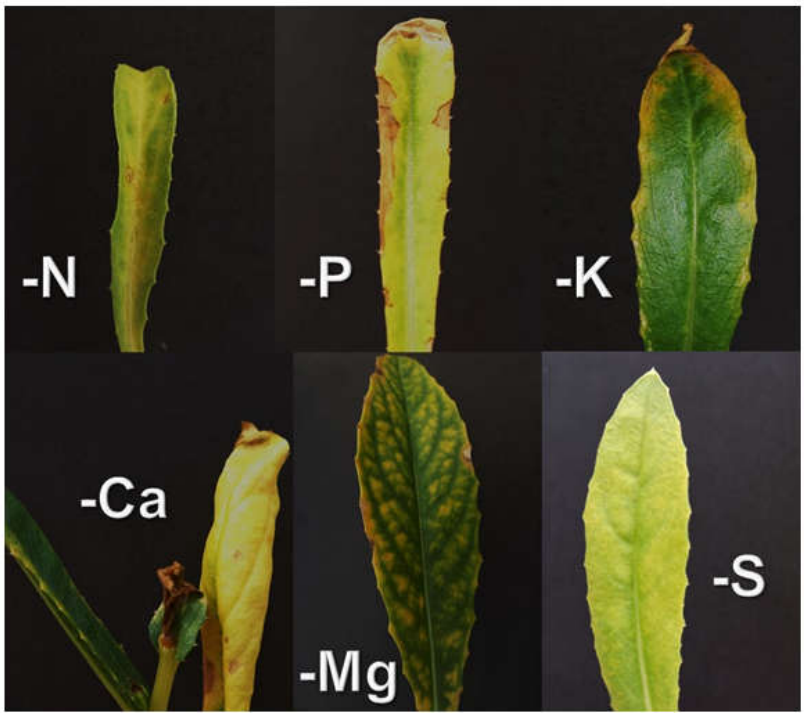

Figure 2. Visual symptoms of safflower plant macronutrient deficiencies in relation to nutrient solution individual omission

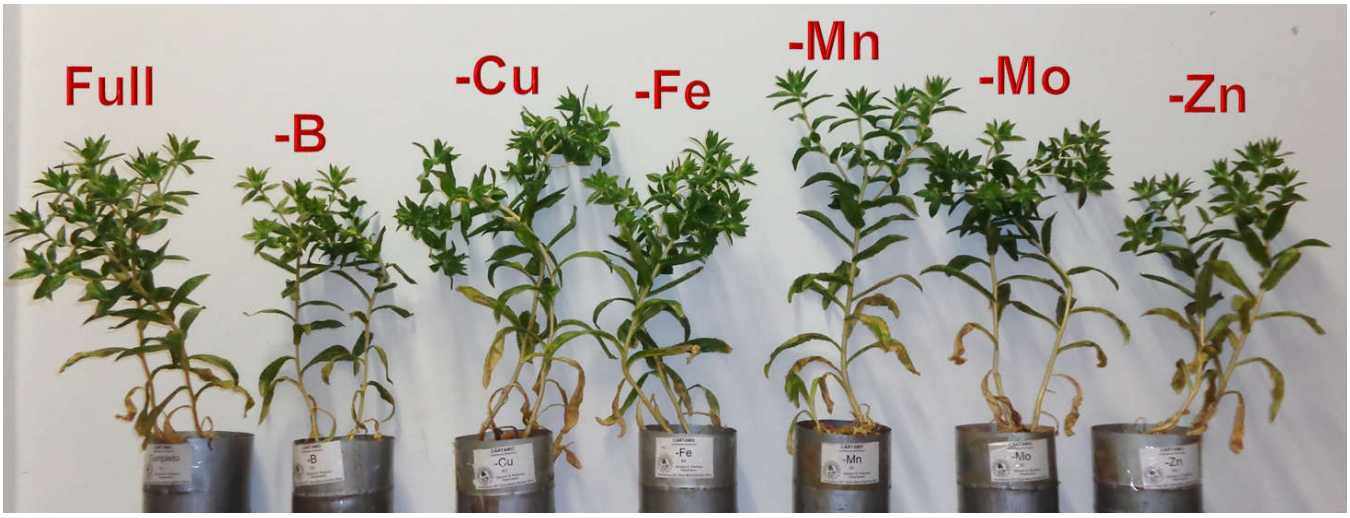

Figure 3. Comparison of safflower plants in relation to complete treatment and micronutrient omission

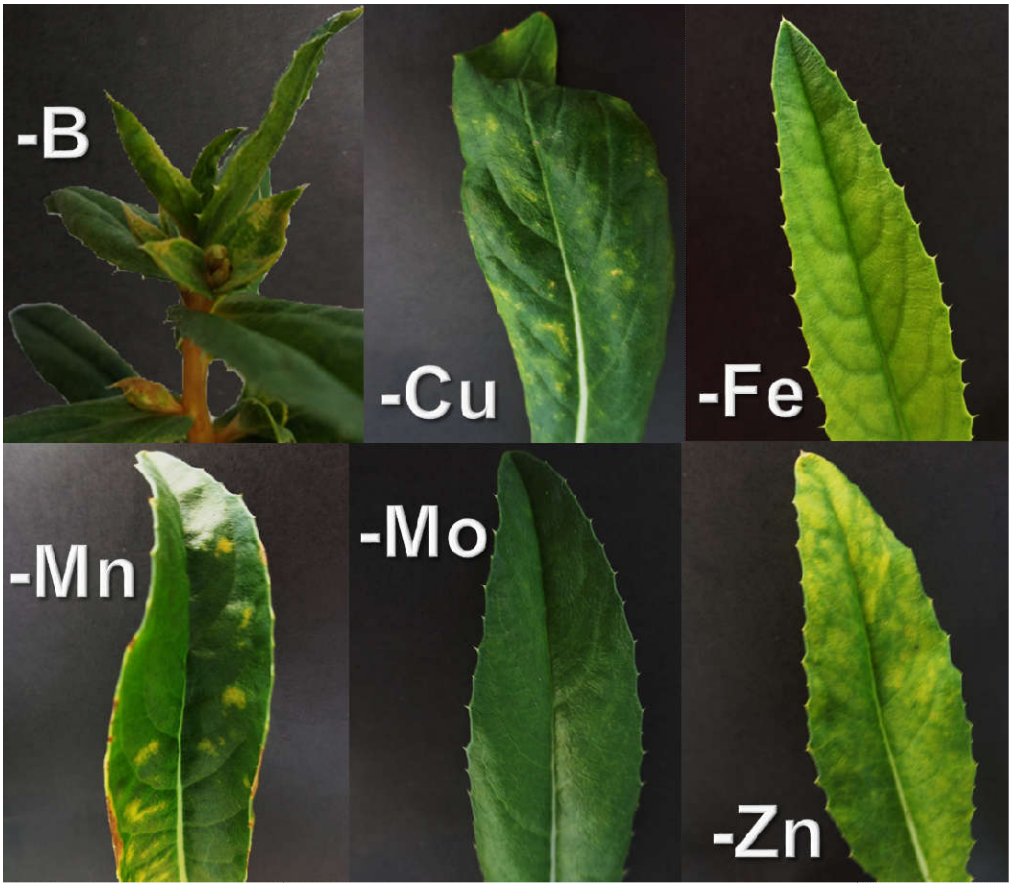

Figure 4. Visual symptoms of safflower plant micronutrient deficiencies in relation to nutrient solution individual omission

the root system, justifying the reduction of development and production characteristics in safflower plants. Lack of calcium in the nutrient solution negatively influenced all variables in safflower plants. In the last evaluation, plants were dead, not producing capitulum (Table 1). Thus, deficiency symptoms in safflower plants were observed, which were expressed at early stages through leaf chlorosis, with apical meristem abscission afterwards (Figure 2). According to Taiz and Zeiger (2013), calcium has low mobility, justifying apical meristem abscission due to not being mobilized for younger tissues.

In addition, calcium is an important cell wall constituent, such as calcium pectates, resulting in plant death before completing the cycle. Magnesium omission to safflower plants influenced plant height and shoot and capitulum dry matter, differing from the complete treatment. However, magnesium was always grouped as having lower influence than other macronutrients, with similar behavior to the omission of some micronutrients (Table 1). Vafaie *et al*. (2013), while studying safflower magnesium fertilization, observed increases in the studied variables compared to fertilization absence, especially for seed

production. Magnesium deficiency symptom was chlorosis distributed in spots through the internerval leaf blade since initial stages. Throughout the crop cycle, chlorosis showed generalization tendency throughout the leaf blade, with limbo spots in capitulum young and peripheral leaves (Figure 2). Visual diagnosis is similar to those found in the study by Prado and Leal (2006), who observed magnesium deficiency in sunflower plants (*Helianthus annuus* L.), which expressed leaf yellowing and leaf blade shriveling.

Those are common symptoms for magnesium deficiency, according to Taiz and Zeiger (2013). Sulfur omission impaired all safflower variables (Table 1). Regarding visual diagnosis, plants showed widespread chlorosis since initial stages for all leaves, including capitulum peripheral leaves (Figure 1 and 2). According to Kumar and Sharma (2013), these symptoms are mainly visible in younger safflower plant leaves, as sulfur has low mobility, differentiating from nitrogen deficiency, which is observed in older leaves. Those results were different from those found by Prado and Leal (2006) in sunflower plants (*Helianthus annuus* L.), who did not found visual symptoms and statistical differences for the variables studied in their research, demonstrating that sulfur is more limiting for safflower than for sunflower crops. For micronutrients, lack of boron and manganese were the ones that differed the most from the complete treatment. Copper and zinc omission significantly reduced capitulum production (Table 1; Figure 3). Boron omission in the nutrient solution caused stem diameter and shoot, root and capitulum dry matter reduction. Boron stood out as the most important micronutrient for safflower plant development (Table 1). Heydarian *et al*. (2012) studied boron foliar application in irrigated safflower plants and observed significant increases for most of their variables, including grain yield, compared with boron application absence. Boron deficiency symptoms were observed in the younger leaves of branches, which had necrosis at their tips and smaller and malformed capitulum (Figure 4).

For Taiz and Zeiger (2013), these symptoms may be related to internal tissue disintegration, as it is a nutrient with low plant mobility, having cell elongation and membrane operation functions. Nutrient solution copper omission did not impair safflower plant structural characteristics. However, it significantly reduced capitulum number and dry matter (Table 1). Copper deficiency caused small yellow spots on the leaf blade of young leaves. Pandey and Sharma (1996) observed visible symptoms and reduced chlorophyll concentration in young safflower leaves with copper deficiency, impairing photosynthesis and plant development. Iron absence was not significantly different from the complete treatment in relation to the analyzed variables (Table 1). According to Jacob-Neto and Rossetto (1998), seed micronutrient reserves are enough for plant development in some species. The symptom caused by iron deficiency in safflower plants was slight internerval chlorosis in younger leaves (Figure 4), corroborating with Kumar and Sharma (2013) regarding safflower plants. According to Taiz and Zeiger (2013), iron is a basic chlorophyll constituent. Its deficiency is expressed by chlorosis in young leaves, as it is not readily mobilized from older leaves. Possibly, iron precipitation occurs in these leaves in form of insoluble oxides and phosphates. Manganese omission differed from the complete treatment in shoot, root and capitulum dry matter and number of capitulum (Table 1). Lewis and McFarlane (1986) and Movahhedy-Dehnavy *et al*. (2009), who studied manganese foliar application in safflower crops, observed production increase. Manganese is important for plant development, as, according to Taiz and Zeiger (2013), one of manganese functions is the photosynthetic reaction in which oxygen is obtained from water. Visual deficiency symptoms observed in safflower plants due to manganese omission were chlorotic spots in the leaf blade and light necrosis in the leaf borders (Figure 4). Molybdenum absence did not influence any development or production variable. Thus, it was not a limiting micronutrient for safflower plants (Table 1). Plants subjected to lack of molybdenum showed no deficiency symptoms in this study (Figure 4). According to Jacob-Netto and Rossetto (1998), in some cases, seed micronutrient reserves, especially molybdenum, are enough to the plant to develop and complete its cycle. In this context, future plans originated from seeds produced under molybdenum deficiency conditions could be impaired. Zinc omission for safflower plants significantly reduced capitulum dry matter (Table 1). Movahhedy-Dehnavy *et al*. (2009) observed increased safflower grain yield with zinc foliar fertilization, while Aytac *et al*. (2014) noted increased yield for different safflower genotypes in relation to fertilization at planting. Zinc omission caused widespread chlorosis in older leaves (Figure 4). For Taiz and Zeiger (2013), zinc deficiency symptoms in plants are expressed in older leaves due to low mobility.

Conclusion

Nitrogen omission, followed by phosphorus omission, caused the highest reductions in safflower development and production. Among secondary macronutrients, calcium omission caused the most significant reductions. In addition, deficiency of these three nutrients (nitrogen, phosphorus and calcium) did not allow for plants to complete their life cycle. Among micronutrients, boron omission, followed by manganese omission, caused the most significant development and production effects on safflower plants. In general, deficiency symptoms were observed in all nutrient absences during visual diagnosis, except for molybdenum omission. Macronutrient omission caused the most severe symptoms compared to micronutrients.

REFERENCES

Abbadi, J. and Gerendás, J. 2012. Phosphorus Use Efficiency of Safflower (*Carthamus tinctorius* L.) and Sunflower (*Helianthus annuus* L.) Studied in Nutrient Solution. *Journal of Agricultural Science and Technology,* 2, 1260-1280. DOI:10.1080/01904167.2014.983115.

Abbadi, J. and Gerendás, J. 2011. Effects of phosphorus supply on growth, yield, and yield components of safflower and sunflower. *Journal of Plant Nutrition*, 34, 1769-1787. DOI:10.1080/01904167.2011.600405.

Abbadi, J., Gerendas, J. and Sattelmacher, B. 2008. Effects of potassium supply on growth and yield of safflower as compared to sunflower. *Journal of Plant Nutrition and Soil Science,* 171, 272-280. DOI: 10.1002/jpln.200700193.

Anicésio, E.C.A., Bonfim-Silva, E.M., Silva, T.J.A. and Koetz, M. 2015. Dry mass, nutrient concentration and accumulation in safflower ('*Carthamus tinctorius*' L.) influenced by nitrogen and potassium fertilizations. *Australian Journal of Crops Science,* 9, 552-560.

Aytac, Z., Gulmezoglu, N., Sirel, Z., Tolay, I. and Torun, A.A. 2014. The effect of zinc on yield, yield components and micronutrient concentrations in the seeds of safflower genotypes (*Carthamus tinctorius* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 42, 202-208.

Bonfim-Silva, E.M., Claudio, A.A., Bär, C.S.L.L., Espirito Santo, E.S. and Pacheco, A.B. 2015. Nitrogênio na produção, índice de clorofila e uso de água no cultivo de rúcula. *Enciclopédia Biosfera*, Centro Científico Conhecer 11, 1386- 1396.

Bonfim-Silva, E.M., Paludo, J.T.S., Sousa, J.V.R., Sousa, H.H.F. and Silva ,T.J.A. 2015. Development of Safflower Subjected to Nitrogen Rates in Cerrado Soil. *American Journal of Plant Sciences* 6, 2136- 2143.

Bonfim-Silva, E.M., Silva, T.J.A., Santos, C.C., Cabral, C.E.A. and Santos, I.B 2011. Características produtivas e eficiência do uso da água em rúcula adubada com cinza vegetal. *Enciclopédia Biosfera*, Centro Científico Conhecer 7, 178-186.

Dordas, C.A. and Sioulas, C. 2008. Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Industrial Crops and Products,* 27, 75-85. DOI: 10.4236/ajps.2015.613215.

Ferreira, D.F. 2008. SISVAR: um programa para análises e ensino de estatística. *Revista Symposium*, 6, 36-41.

Haghighati, A., 2010. Study on the effects of nitrogen and phosphorus fertilizers on the yield and oil content of safflower lines in Drylands. *Research Journal of Agronomy* 4, 57-62. DOI:10.3923/rjagr.2010.57.62.

Heydarian, M., Moghadam, H.R.T., Hassanpour, J. and Zahedi, H. 2012. Effect of boron foliar application and irrigation withholding on yield and yield components of safflower. *Research on Crops,* 13, 166-173.

Hoagland, D. and Arnon, D.I. 1950. The water culture method for growing plants whithout soil. *California Agriculture Experimental Station,* 347, 1-32.

Jacob-Neto, J. and Rossetto, C.A.V. 1998. Concentração de nutrientes nas sementes: o papel do molibdênio. *Floresta e Ambiente* 5, 171-183.

Kulekci, M., Polat, T. and Ozturk, E. 2009. The determination of economically optimum nitrogen dose in safflower production under dry conditions. *Bulgarian Journal of Agricultural Science* 15, 341-346.

Kumar, P. and Sharma, M.K. 2013. 'Nutrient Deficiencies of Field Crops: Guide to Diagnosis and Management.' pp. 400 (CABI: Boston, USA)

Lewis, D.C. and McFarlane, J.D. 1986. Effect of foliar applied manganese on the growth of safflower (*Carthamus tinctorius* L.) and the diagnosis of manganese deficiency by plant tissue and seed analysis. *Australian Journal of Agricultural Research* 37, 567-572. DOI: 10.1071/ AR9860567.

Martin, W., Sailsbery, R., Brandon, M. and Petersen, R. 1971. Answering the riddle of poor safflower after rice … banded phosphorus may be the solution. *California Agriculture* 25, 4-6.

Movahhedy-Dehnavy, M., Modarres-Sanavy, S.A.M. and Mokhtassi-Bidgoli, A. 2009. Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. *Industrial Crops and Products* 30, 82-92. DOI:10.1016/j.indcrop.2009.02.004.

Pandey, N. and Sharma, C.P. 1996. Copper effect on photosynthesis and transpiration in safflower. *Indian journal of experimental biology* 34, 821-822.

Porto, R.A., Bonfim-Silva, E.M., Souza, D.S.M., Cordova, N.R.M., Polyzel, A.C. and Silva, T.J.A. 2013. Adubação potássica em plantas de rúcula: produção e eficiência do uso da água. *Revista Agroambiente On-line* 7, 28-35.

Prado, R.N. and Leal, R.M. 2006. Desordens nutricionais por deficiência em girassol var. Catissol-01. *Revista Pesquisa Agropecuária Tropical* 36, 187-193.

Taiz, L. and Zeiger, E. 2013. 'Fisiologia vegetal' 5 ed (Artmed: Porto Alegre, Brasil)

Taleshi, K., Shokoh-Far, A., Rafiee, M., Noormahamadi, G. and Sakinejhad, T. 2012. Safflower Yield Respond to Chemical and Biotic Fertilizer on Water Stress Condition. *World Applied Sciences Journal* 20: 1472-1477. DOI:10.5829/idosi.wasj.2012.20.11.1733.

Vafaie, A., Ebadi, A., Rastgou, B. and Moghadam, S.H. 2013. The effects of potassium and magnesium on yield and some physiological traits of Safflower (*Carthamus tinctorius*). *International Journal of Agriculture and Crop Sciences*, 5, 1895-1900.

Vincent, J.M. 1970. *Manual for the Practical Study of Root Nodule Bacteria*. 1 ed. (Blackwell: Oxford, Egland).

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