Changes in some physiological traits and mucilage yield of sour tea (*Hibiscus sabdariffa* L.) under foliar application of magnesium and iron oxide nanoparticles

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Abstract

In order to investigate the effect of magnesium oxide (MgO) and iron oxide (Fe_2O_3) nanoparticles (NPs) on physiological traits and the yield of sour tea (*Hibiscus sabdariffa* L.), a factorial experiment was conducted in a randomized complete block design (RCBD) with four replications in 2018. MgO and Fe_2O_3 were used in concentrations of 0.01% and 0.03% as foliar application. The results showed NPS improved chlorophyll (Chl) content and index. The highest total chlorophyll was obtained in plants treated with 0.01% of MgO and 0.0.3% of Fe_2O_3. Catalase (CAT) and peroxidase (POX) activities significantly increased with NPs, in which all combined concentrations of NPs had greater enzymes activities compared to control. The seed weight in plants treated with 0.03% of MgO and 0.01% of Fe_2O_3 was higher compared to non-NPs treatment. The highest mucilage yield was found at 0.01% of MgO and 0.03% of Fe_2O_3. Total flavonoid content (TFC) in plants treated with 0.01% of MgO and 0.01% of Fe_2O_3 was higher than other experimental treatments. The treatment including 0.01% of MgO and non Fe_2O_3 had lower anthocyanin content compared to other combined levels of NPs. Carotenoid increased with medium concentrations of NPs, but decreased with high level of NPs (0.03%). Protein concentration at all combinations of 0.1% and 0.3% NPs was higher than other experimental treatments. According tour results, we suggest the use of 0.01% of MgO and 0.03% of Fe_2O_3 for improving the physiological properties of sour tea.

Keywords: *Iron oxide; Magnesium oxide; Nanoparticle; Physiological status.*

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Introduction

Recently the use of plant products instead of chemical materials is one of the most important needs of modern civilization in terms of human health (Mirzaei et al., 2020; Ruwali and Negi, 2019). Sour Tea (*Hibiscus Sabdarifa*), which is belonging to the Mallow (*Malva*) family, is widely cultivated in tropical and semi-arid regions (Mohamed et al., 2012). Sepal of this plant is the most important economic organ that is widely used as a flavoring agent for jam, jelly, gelatin, syrup, ice cream, pudding and cake (Hadi et al., 2017). Sour tea is rich in secondary metabolites

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Table 1
Physical and chemical properties of experimental field soil

Depth	texture	EC (ds m^{-1})	pH	O.C (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Fe (mg kg^{-1})	Mg (mg kg^{-1})
0-30	Loam	2.96	7.8	0.91	0.08	12.7	196	18.2	37.1

with medicinal properties. It also contains a large amount of organic acids (citric, oxalic, tartaric acid) and vitamin C and anthocyanins (Mohamed et al., 2012).

Among the macro elements of plants, magnesium (Mg) as the central nucleus of chlorophyll, is responsible for a number of plant yield and as activating enzymatic systems controlling photosynthetic processes, energy transfer and the production of carbohydrates, proteins and lipids (Gransee and Führs, 2013). Therefore, the deficiency of this element reduces the quantitative and qualitative performance of plants (Gransee and Führs, 2013). Iron (Fe) plays an important role in metabolic activities such as nitrogen fixation, chlorophyll and thylakoid production, chloroplast development, pigment production and as a catalyst, is involved in enzymatic activities such as respiratory tract enzymes and glycolate. At Fe deficiency conditions, the amount of photosynthesis and the rate of fixation of carbon dioxide per leaf area will be decreased, which results in reduced performance (Rizwan et al., 2019).

(Nasiri and Najafi, 2015)Nano-fertilizers due to their high performance result in increased nutrimental utilization efficiency and soil toxicity reduction. In recent years, the effect of solubility of plant-based elements in the form of nanoparticles (NPs) on growth and yield of plants has been widely considered. Therefore, nutritional management of these elements can be effective in the production of high quality of medicinal and aromatic herbs. Previous studies have shown the effective effect of Mg and Fe of plant growth and yield. Delfani et al. (2014) showed positive effects of Fe and Mg nanofertilizers on physiological characteristics of black-eyed pea. An increase in leaf protein, chlorophyll, and carotenoid contents of spinach were reported when Mg was used as foliar application (Borowski and Michalek, 2010). Rui et al. (2016) revealed the improvement of growth and yield of peanut in plants treated with iron oxide NPs (Rui et al., 2016). The improvement in essential oil quantity and quality was obtained with as foliar application of Fe and Zn (Nasiri and Najafi, 2015). There is no document for foliar application of Fe and Mg on physiological status of sour tea. Therefore, the preset study was carried out to evaluate the effect of foliar application with Fe and Mg on photosynthesis content, enzyme activities, flavonoid and anthocyanin content, and mucilage yield of sour tea.

Materials and Methods

Site description

The present study was carried out in the research farm of Hashtgerd (1210 m asl, 35°58´45" N, 50°45´30"E), Iran. At the beginning of the experiment, a compound of the surface (0–30 cm) soil samples was collected, air-dried, passed through a 2-mm sieve and the soil physical and chemical properties were measured (Table 1). During the study period mean maximum temperature fluctuated from 17.3 °C to 28.2 °C; whereas; mean minimum temperature varied from 2.6 °C to 15.4 °C. The most precipitation occurred in March (43.2 mm) and April (39.1 mm) (Fig. I).

Experimental treatments and design

In order to investigate the effect of magnesium oxide and iron oxide nanoparticles on physiological traits and yield of sour tea, a field experiment was conducted in Hashtgerd as factorial based on a completely randomized block design (CRBD) with four replications in 2018. MgO and Fe2O3 were used in different concentrations of 0.01 and 0.03%. The land was prepared by performing a plow and two perpendicular disks before planting. Each experimental unit consisted of 6 planting lines in 8 m long, spaced 70 cm from each other. Before planting, soil sample (0-30 cm depth) was sent to the Laboratory of Water and

Soil to determine the soil physicochemical properties (Table 1).

Seeds of sour tea were purchased from Pakan Bazr Company® and then cultivated at intervals of 30 cm on a row at 24th to 26th May 2018.

Chlorophyll (Chl) index

To determine Chl index, four random terminal and side plants in each experimental treatment were tagged and their final growth was measured using a SPAD meter (Minolta-502 model). Chl index between 10 am and 2 pm were determined using equipment based on a new technology (Mahdavi et al., 2017).

Chlorophyll (Chl) content

To measure the content of total Chl., 200 mg of fresh samples were homogenized in 8 ml 80% acetone. After that, the mixture was centrifuged at 4 °C for 15 min (3000 rpm). Supernatants were used for analyzing chlorophyll content. Absorbance was determined at 645 and 663 nm by the spectrophotometer (Arnon, 1949).

Protein extraction

For total protein determination, 0.8 g fresh leaves were homogenized at 4 °C in 1 M Tris–HCl (pH 6.8) using a mortar. The homogenates were centrifuged twice at 4 °C for 30 min at 13,000g using a Heraeus 400R microfuge. The supernatants were kept at −70 °C until protein determination and enzyme assay. Protein concentration was determined according to Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as standard. The absorbance was measured at 595 nm by UV–visible spectrophotometer (UV-160, Shimadzu, Tokyo, Japan).

Catalase (CAT) activity

The measurement of initial rate for H_2O_2 disappearance was applied to measure CAT activity (Bergmeyer, 1970). The reaction mixture contained 3% H_2O_2 and 0.1 mM EDTA in 0.05 M Na–phosphate buffer (pH 7). The reduction in H_2O_2 concentration was determined as a decline in optical density at 240 nm by UV–visible spectrophotometer (UV-160, Shimadzu, Tokyo, Japan) and the activity was measured as µmol H_2O_2 decomposed per minute.

Peroxidase (POD) activity

Peroxidase activities were quantified based on Herzog description (Herzog, 1973). The reaction mixture composed of 4 ml of 0.2 M acetate buffer (pH 4.8), 0.4 ml H_2O_2 (3 %), 0.2 ml of 20 mM benzidine and 30 µl enzyme extract. Absorbance of the reaction solution was measured at 530 nm by UV–visible spectrophotometer (UV-160, Shimadzu, Tokyo, Japan). The POD activity was specified as 1 µM of benzidine oxidized per min per mg protein [Unit mg^{-1} (protein)].

Determination of total flavonoid content (TFC)

The flavonoid levels were measured by aluminum chloride colorimetric method (Zhishen et al., 1999). Briefly 0.5 ml of extract solution with 1.5 ml of 95% ethanol, 0.1 ml of aluminum chloride 10%, 0.1 ml of 1 M potassium acetate were mixed with 2.8 ml of distilled water. The mixture vortexed for 10 s and left to stand at 25 °C for 30 min. The absorbance of the mixture was read at 415 nm. Quercetin concentrations (0 to 1200 µg ml^{-1}) were prepared and linear fit was used for calibration of the standard curve.

Determination of anthocyanins

Leaf discs (1 cm in diameter) were selected to assay anthocyanins concentration in acidified methanol (HCl:methanol, 1:99, v/v) as determined by (Havaux and Kloppstech, 2001). Absorption spectra of the extracts were determined at 530 nm after centrifugation (4000 rpm for 10 min) using a UV-1700 spectrophotometer (Shimadzu, Tokyo, Japan).

Mucilage yield

The amount of mucilage of sepal sprays of each treatment was measured and the mucilage yield of each treatment was obtained by multiplying the

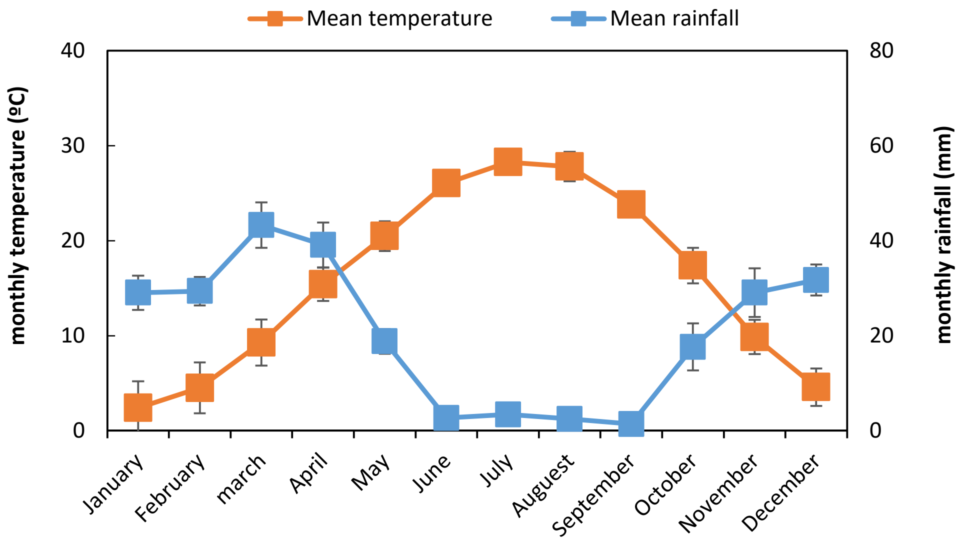

Fig. I. The climate conditions of Hashtgerd during 2018 (National Meteorological organization, Iran).

amount of mucilage in the amount of dry matter yield (Kalayasundram et al., 1982).

Statistical analysis

All data were analyzed statistically by analysis of variance using the software SAS version 9.3 (Cary, NC: SAS Institute Inc., 2011). The means separated using the Duncan test at P < 0.05 level.

Results

Chlorophyll (Chl)

Total Chl significantly improved with both MgO and Fe_2O_3. All concentrations of NPS improved chlorophyll content. The highest total chlorophyll was obtained in plants treated with 0.01% of MgO and 0.0.3% of Fe_2O_3 to be 2.10 mg g^{-1} FW. 0.01% of MgO and 0.0.1% of Fe_2O_3 increased total Chl content by 1.5 fold. For Chl index, 0.01% of MgO and 0.03% of Fe_2O_3 was significantly higher compared to non-NPs treatment (Table 2).

Enzyme activities

CAT activity significantly increased with NPs. For all concentrations of NPs, we obtained greater activities of compared to non-NPs application. The high CAT activity was found in plants treated with simultaneous application of MgO and Fe_2O_3. In addition, POX activity increased with NPs application. The maximum activity of POX was observed at 0.01% of MgO and 0.03% of Fe_2O_3 to be 232.4 U mg^{-1} protein (Table 2).

Seed weight and yield

Seed weight was significantly increased with NPs application. The seed weight in plants treated with 0.03% of MgO and 0.01% of Fe_2O_3 increased by 17% compared to non-NPs treatment. All levels of NPs improved seed yield. In plants treated with 0.01% of MgO and 0.03% of Fe_2O_3, seed yield improved by 24% in comparison with non-NPs treatment. (Table 3).

Mucilage yield

Mucilage yield was significantly changed when NPs was used. The highest mucilage yield (185.4 kg ha^{-1}) was found at 0.01% of MgO and 0.03% of Fe_2O_3. In simultaneous application of the high level of NPs (0.03%), we observed a significant decline of seed yield compared to other concentrations of NPs (Table 3).

Soluble protein content

Protein was significantly influenced with foliar application of NPs. Protein concentration at 0.01% of MgO and 0.03% of Fe_2O_3 (1.40 mg g^{-1} FW) and 0.03% of MgO and 0.01% of Fe_2O_3 (1.29 mg g^{-1} FW)

Table 2
Chlorophyll content and index, and enzyme activities of sour tea under foliar application of MgO and Fe_2O_3

MgO	Fe_2O_3	Total Chl (mg g^{-1} FW)	Chl index (mg g^{-1} FW)	CAT (U mg^{-1} protein)	POX (U mg^{-1} protein)
0	0	0.71 ± 0.01^{g}	36.9 ± 2.5^{b}	19.4 ± 0.08^{e}	79.1 ± 15.9^{e}
0	0.01 %	0.88 ± 0.03^{ef}	38.1 ± 1.8^{ab}	25.2 ± 0.28^{c}	91.2 ± 8.1^{e}
0	0.03 %	0.98 ± 0.02^{d}	39.7 ± 0.3^{ab}	26.1 ± 0.24^{bc}	160.3 ± 15.8^{c}
0.01 %	0	0.91 ± 0.01^{ed}	39.5 ± 0.2^{ab}	24.9 ± 0.03^{c}	140.0 ± 15.3^{d}
0.01 %	0.01 %	1.80 ± 0.04^{b}	41.1 ± 2.8^{ab}	27.2 ± 0.19^{b}	176.0 ± 2.5^{c}
0.01 %	0.03 %	2.10 ± 0.01^{a}	41.5 ± 3.5^{a}	33.8 ± 0.07^{a}	232.4 ± 9.1^{a}
0.03 %	0	1.07 ± 0.01^{c}	38.2 ± 2.1^{ab}	26.4 ± 0.31^{bc}	165.0 ± 2.6^{c}
0.03 %	0.01 %	1.87 ± 0.02^{b}	41.0 ± 2.7^{ab}	32.9 ± 0.24^{a}	198.7 ± 2.2^{b}
0.03 %	0.03 %	0.81 ± 0.01^{f}	39.1 ± 0.6^{ab}	21.8 ± 0.14^{d}	87.0 ± 2.45^{e}

Small letters in each column show the significance of mean comparison by Duncan multiple range test ($P \leq 0.05$).

Table 3
Seed weight and yield, and mucilage yield of sour tea under foliar application of MgO and Fe_2O_3

MgO	Fe_2O_3	1000 seed weight (g)	seed yield (kg ha^{-1})	Mucilage yield (kg ha^{-1})
0	0	18.0 ± 0.14^{b}	320.6 ± 4.05^{c}	83.2 ± 5.89^{c}
0	0.01 %	18.6 ± 2.25^{ab}	338.2 ± 22.8^{bc}	121.9 ± 15.4^{bc}
0	0.03 %	18.7 ± 1.81^{ab}	$355.9 \pm 4.08^{a-c}$	126.3 ± 2.04^{bc}
0.01 %	0	19.2 ± 0.07^{ab}	337.9 ± 1.19^{bc}	116.4 ± 5.65^{bc}
0.01 %	0.01 %	20.2 ± 1.14^{ab}	378.8 ± 6.05^{ab}	135.4 ± 14.95^{bc}
0.01 %	0.03 %	21.3 ± 1.84^{a}	398.6 ± 82.05^{a}	185.4 ± 25.7^{a}
0.03 %	0	19.0 ± 2.26^{ab}	$367.2 \pm 1.09^{a-c}$	124.7 ± 3.17^{bc}
0.03 %	0.01 %	21.2 ± 0.23^{a}	379.6 ± 21.3^{ab}	159.5 ± 18.6^{ab}
0.03 %	0.03 %	18.1 ± 0.06^{b}	323.1 ± 1.19^{c}	90.5 ± 21.8^{c}

Small letters in each column show the significance of mean comparison by Duncan multiple range test ($P \leq 0.05$).

was higher than other experimental treatments (Fig. II).

Total flavonoid content (TFC) and anthocyanin

TFC in plants treated with 0.01% of MgO and 0.01% of Fe_2O_3 was higher than other experimental treatments. We found medium concentration of NPs is appropriate in increasing the TFC of sour tea (Fig. III). Anthocyanin was changed with some concentrations of NPs. The treatment including 0.01% of MgO and non Fe_2O_3 had lower anthocyanin content compared to other combined levels of NPs (Fig. IV).

Discussion

Foliar application with MgO and Fe_2O_3 significantly improved the physiological status of sour tea. The critical limit of the Fe in soil for most plants is 16-10 mg kg^{-1} soil, and for Mg is 74 mg kg^{-1} soil. According to our soil analysis, the obtained Fe and Mg concentrations are lower than the critical values. Therefore, to compensate the deficiency of the elements, we used both NPs. It is well documented that NPs compounds due to their small size and high solubility are rapidly absorbed by plants and eliminate food shortages and plant requirements (Boxall et al., 2007)

Chl index shows a positive correlation with leaf Chl content (Gerendás and Führs, 2013). It seems that increasing the Chl content with NPs application can be due to the role of both Mg and Fe in the activation of the protein syntheses of the pathway of biosynthesis of chlorophyll and some antioxidant enzymes such as CAT and POX in protecting Chl from degradation by reactive oxygen species (ROS) (Delfani et al., 2014). Also, the common substance for the production of chlorophyll is the delta-aminoleulinic acid, which is inhibited by the formation of iron. The use of iron or Mg as the central atom within tetrapyrrole leads to the formation of coenzyme and magnesium-protoporphyrin, respectively. It has been shown that iron is required for the formation

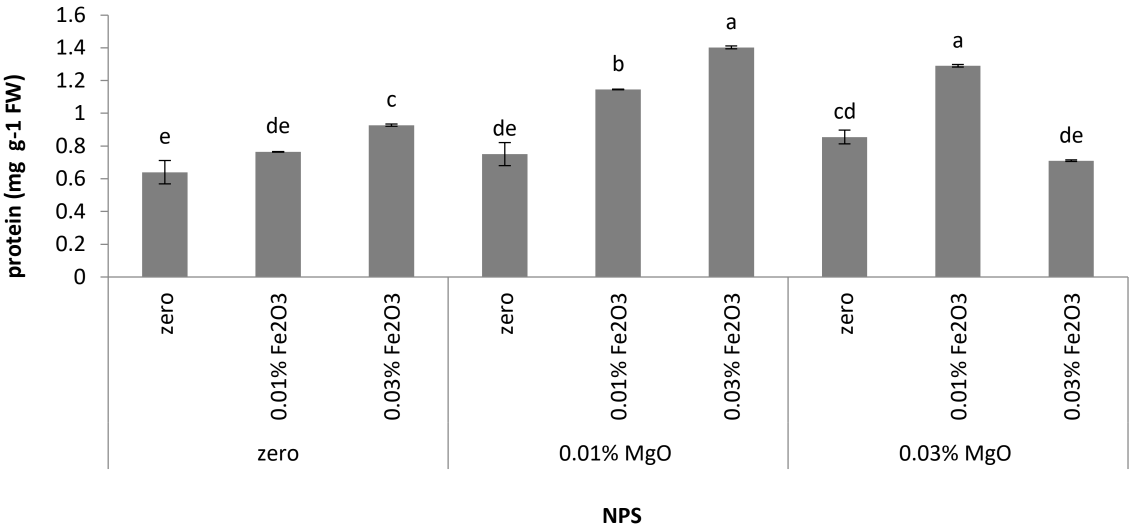

Fig. II. Protein content of sour tea under foliar application of MgO and Fe_2O_3

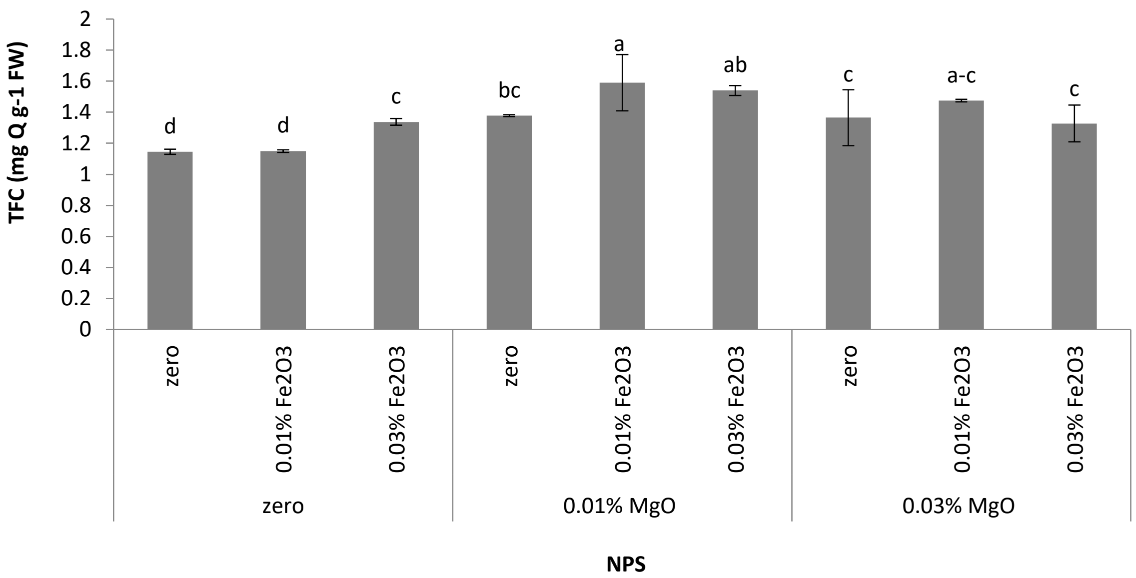

Fig. III. Total flavonoid content (TFC) of sour tea under foliar application of MgO and Fe_2O_3

of protochlorofilide from magnesium-protopurephrin. Likewise, the coproporphyrinogen oxidase enzyme, which is a fermentative protein, catalyzes magnesium-protoporphyrin oxidation to proto-chlorophyllide. An increase in the Chl content under Fe and Mg application was reported in almond (Rui et al., 2016) and barley (Tränkner et al., 2016).

Protein content increased with NPs. Borowski and Michałek (2010) indicated the positive response to Mg fertilizer on spinach. Ribosomes are macromolecular structures that are responsible for the biosynthesis of proteins from proteins and ribonucleic acids. The structure of ribosomes consists of two subunits, the Mg is located in the form of a bridge between the two subunits, and if this element is deficient, the ribosomal structure is broken up and thus the protein synthesis is not performed. On the other hand, the application of Mg increases the nitrogen stabilization and absorption by the root, and increase nitrogen efficiency, and plays a role in protein synthesis (Gerendás and Führs, 2013). Also, Fe2O3 increased the protein production of this plant, probably due to the strengthening of the oxidation system and regeneration and

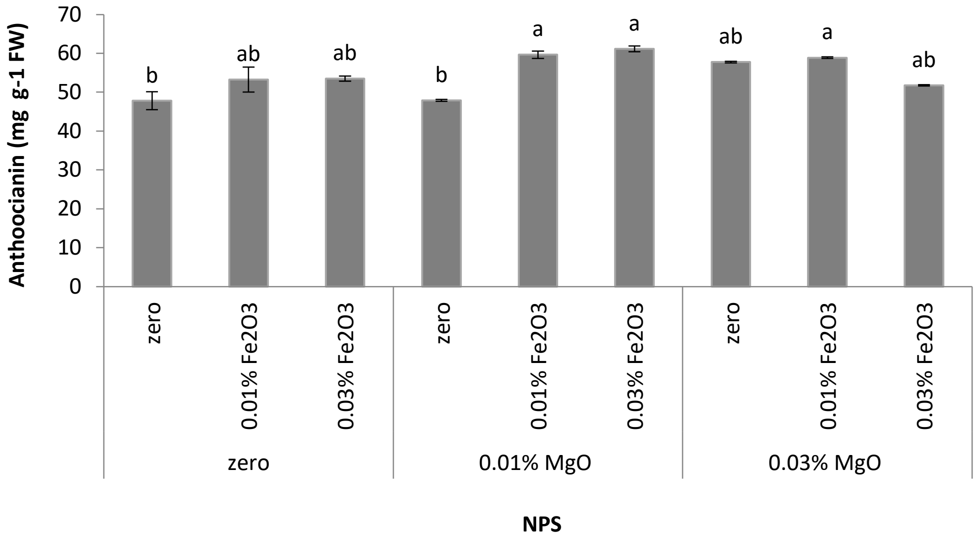

Fig. IV. Anthocyanin concentration of sour tea under foliar application of MgO and Fe_2O_3

activation of enzymes and oxygen carriers in nitrogen stabilization by the Fe (Gerendás and Führs, 2013). Similar to this work, Delfani et al. (2014) reported an increase if protein content of black-eyed pea due to foliar application of Mg and iron.

Flavonols are found in higher plants. They are products of the flavonoid biosynthetic pathway (Mattivi et al, 2006), which mainly define the antioxidant potential of plants (Portu et al., 2015). Our results seem to indicate that Fe application had less influence on anthocyanin composition than on TFC compounds. Fe deficiency induced leaf chlorosis leading to reduced photosynthetic efficiency and electron transport, with less carbon being fixed via photosynthesis. The improvement of Fe treatment on photosynthesis efficiency could influence the way the vine uses the precursors for the synthesis of phenolic compounds or other secondary metabolites. Similarly, it was reported that strawberry fruits produced under Fe deficiency showed higher concentrations of anthocyanins, benzoic acids and flavonols (Valentinuzzi et al., 2015). Foliar application of Fe improved TFC of grape berry (Shi et al., 2018).

Seed yield increased with the NPs application. Due to the lack of Mg in most soils, its transfer from root to aerial parts of plants is impaired. Therefore, the application of Mg not only prevents injuries due to Mg deficiency, which in some way leads to a reduction in the yield of the sour tea, but also increases the yield of the seed of this plant. Also, regarding the effect of foliar application of Fe on seed yield, it should be noted that Fe in the photosynthesis process plays an important role in the oxidation and reduction processes due to the presence of containing proteins such as cytochromes and ferredoxins (Delfani et al., 2014). Rui et al. (2016) showed an increment of seed yield with Fe by increasing the levels of phytohormones.

Considering that an increase in the mucilage yield of the sour tea plants depends on the sepal yield and the percentage of mucilage, it can be stated that the plants supplied with Fe and Mg have higher mucilage yield due to increase in the quality of photosynthesis caused by photosynthesis and increase of carbohydrates (Mahdavi et al., 2017). Accordingly, it was shown an improvement of the foliar application of nano-iron chelates on the mucilage yield of *Plantago psyllium* (Aghazadeh-Khalkhali et al., 2015).

Conclusions

The results showed that foliar application on sour tea with Fe2O3 and MgO fertilizers prepared by nanotechnology increased the physiological status and mucilage yield of the medicinal plan. Foliar application of different concentrations of the NPs was different in most traits. However, the use of 0.01% of one NP along with 0.03% of another NP is more effective compared to other treatments. High concentrations (0.03%) of NPs in the reciprocal effect conditions, by increasing the production of radical's toxoid release and oxidative stress induction are a factor in inhibiting growth and causing symptoms of toxicity in the sour tea.

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