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ORIGINAL PAPER

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# EFFICIENCY OF DIFFERENT METHODS AND FORMS OF MICROELEMENTS APPLICATION IN FUNCTION OF N FERTILIZER IN APPLE TREES

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## ABSTRACT

In order to achieve a high yield and quality of apple fruit, more effective ways of fertilization are required in the modern, high density apple orchards. The objective of this research was to determine the efficiency (partial nutrient balance, PNB) of different methods (foliar and fertrigation) and forms (chelates and salts) of microelements application in relation to the levels of N fertilization in apple orchard cultivar ('Golden Delicious'). The combined effects of these fertilizers on the number of apple fruits per tree and on the yield per tree were also studied. Foliar application of Mn, Zn and Fe had significantly higher partial nutrient balance values compared to the soil application in both years of the experiment. However, most of the PNB values were below 10% indicating relatively low efficiency of the applied fertilizers with microelements.

**Key words:** foliar, fertigation, micronutrients, nitrogen, partial nutrient balance

## INTRODUCTION

A proper cultivation of apples requires an adequate amount and application method of fertilizers for their normal growth and development. Also, the rational and timely use of fertilizers is one of the basic conditions for achieving high quality and economically viable crops. Intensive methods of orchard cultivation require more efficient ways of irrigation and fertigation. However, the application of microelements through the soil is generally considered to be less effective in relation to the foliar application due to adsorption of microelements in soil [Neilsen and Hoyt 1990]. On the other hand, there is a lack of studies in which the influence of foliar and the application of microelements by fertigation are compared.

Nitrogen fertilization of apple trees has great influence on tree growth, yield and fruit quality [Neilsen and Neilsen 2002, Raese et al. 2007]. Besides that, nitrogen fertilization could have positive impact on concentration of microelements in plant tissues due to increased synthesis of nitrogenous organic compounds (phytosiderophores) which plants excrete through the roots and have the role of mobilizing metals (microelements) in soil due to the formation of chelate complexes [Waters et al. 2006, Suzuki et al. 2008, Borg et al. 2009]. Also, the application of nitrogen fertilizers facilitates the adoption of microelements by the root of plants due to the positive effect on the number of protein transporter in root cell membranes, as well as the

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facilitated transport of microelements through xylem and floam [Waters et al. 2006, Borg et al. 2009, Palmer and Guerinot 2009].

Iron (Fe), manganese (Mn) and zinc (Zn) are essential micronutrients in plant nutrition, needed in rather small amounts but playing indispensable physiological roles in enzyme production, metabolic processes, and photosynthetic production [Khoshgoftarmanesh 2010, Dimkpa and Bindraban 2016]. Their role for plants growth and development dictates the careful selection of methods and chemical compounds used for fertilization [Murtić et al. 2012]. Selecting the adequate fertilizers should be in function of soil condition, since the micronutrients may become unavailable in soils with a high pH [Mengel 1994], and according to their capacity of penetrating leaf cuticles [Post-Beittenmiller 1996].

Apple production is highly profitable and gets more importance in Serbia which results in apple orchards expansion on very productive soils such as Chernozem. Chernozem is a soil of the semiarid steppe areas, located in Northern Serbia on an area of about 1,000,000 ha. Parent material is calcareous loess, aeolian sediment with 20–30% calcium carbonate ($CaCO_3$). On the smaller areas Chernozem was created on the redeposited loess, alluvium and aeolian sand. Besides the high productivity, Chernozem in Serbia is mostly slightly to the high alkaline soil. Given that elevated soil pH has negative impact on micronutrients availability in soil, symptoms of Fe, Zn and Mn in apple production occurs relatively often.

Nitrogen fertilization may be a significant factor influencing the yield and apple tree vigor, which may further affect the result of micronutrients application. The aim of this research was to examine the effects of different methods and forms of micronutrients (Fe, Zn and Mn) application and N fertilization, as well as their interaction in relation to yield and micronutrients uptake by apple tree ‘Golden Delicious’.

## MATERIAL AND METHODS

The experiment was performed in Serbia on the experimental fields of the Faculty of Agriculture, University of Novi Sad (45°20'24.4"; 19°5'22.3"E) in 2014 and 2015. Three and four years old ‘Golden Delicious Reinders’® trees were used in the experiment. The trees were on M9 T337 rootstock, planted at a 3.2 × 0.8 m distance. The ‘Golden Delicious’ plant was fertilized using various levels of N fertilizers and different ways of application. The experiment was conducted in a two-factor split-plot design in order to determine the changes in the micronutrient contents in apple leaves and fruits as well as the number of apple fruits per tree, average fruit weight and total yield.

On the main plots, the effect of N application was studied involving three fertilization rates of 0, 80, 160 kg N $ha^{-1}$ in the form of ammonium nitrate ($NH_4NO_3$, 34.4% N). The subplots included the application of micronutrients using foliar fertilizers in chelated form –DTPA, and in the form of salts (sulfates), while the proportion of each individual active substance was as follows: 1.5 kg Fe $ha^{-1}$; 0.5 kg Mn $ha^{-1}$; 0.5 kg Zn $ha^{-1}$, and by fertigation in which the proportion were: 4.5 kg Fe $ha^{-1}$; 1.5 kg Mn $ha^{-1}$; 1.5 kg Zn $ha^{-1}$. Nitrogen was applied in the first half of vegetative growth of apples. The micronutrients were applied two times during intensive shoots growth. The first application was carried out at a when shoots reach the length of 20 cm, and the second application was 15 days after the first. The drip irrigation system was operated periodically during the period April–September (in each year) to maintain soil moisture tension between 15 and 25 kPa (measured by a tensiometer with the ceramic tip at 15 cm below the surface of the bed between two plants in a row) in all experimental plots. The total monthly precipitation and average air temperature during the experiment are given in Figure 1.

The year 2014 is characterized by a significantly higher rainfall compared to the long-term average (LTA), especially in the first half of apple vegetation (Figure 1). The precipitation rate in April was almost four times higher (by 142 mm) than LTA, so total amount of water used for drip irrigation was 80 l $m^{-2}$, while in 2015 the annual irrigation norm was 170 l $m^{-2}$.

The experiment was set on the Chernozem soil with the following chemical properties: pH ($H_2O$), 8.5; pH (KCl), 7.4; $CaCO_3$, 4.2%; organic carbon (C), 2.01%; $P_2O_5$, 69.30 mg $kg^{-1}$; $K_2O$, 232.82 mg $kg^{-1}$; Mg 145 mg $kg^{-1}$; Fe 12.21 mg $kg^{-1}$; Mn 24.33 mg $kg^{-1}$; Zn 1.92 mg $kg^{-1}$.

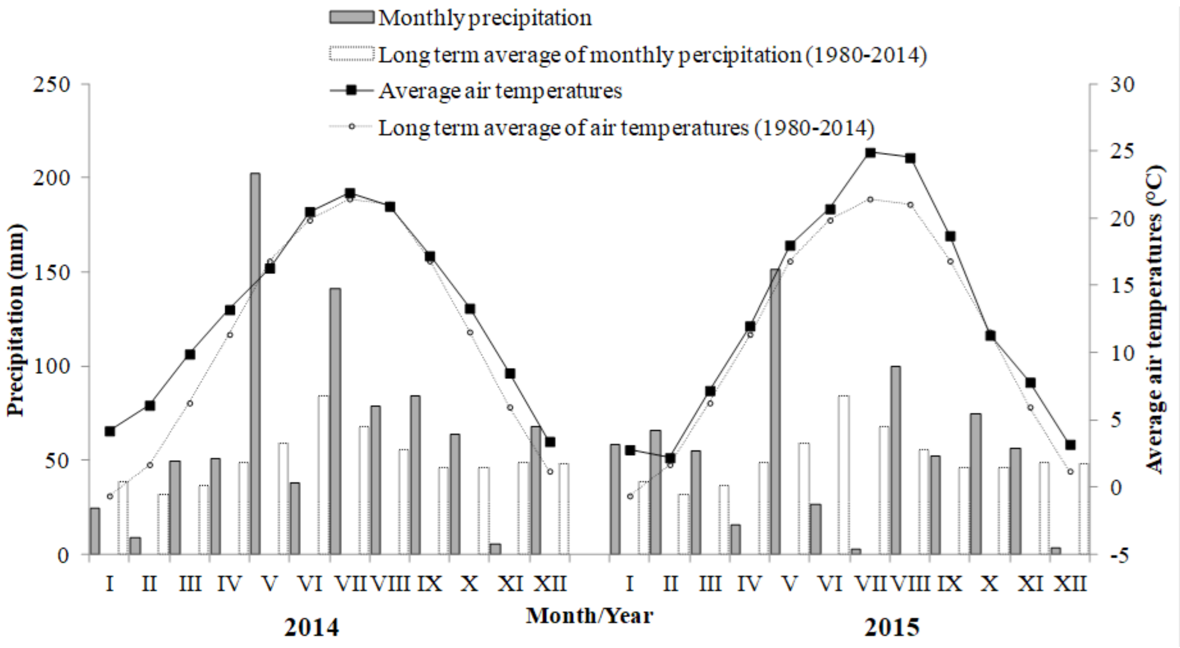



**Fig. 1.** Total monthly precipitation and average air temperature (2014–2015)

The pH value of the soil was determined in 1 : 2.5 suspension of soil : water and soil : 1 M KCl (Mettler Toledo, Five Easy FE 20). The content of $CaCO_3$ was determined volumetrically using a Scheibler calcimeter (Hedas, Serbia). Organic carbon was determined by Tyurin's method [ISO 14235:2005]. Extractable phosphorus ($P_2O_5$) and potassium ($K_2O$) were extracted with a solution of 0.1 M ammonium lactate and 0.4 M acetic acid, at a soil : solution ratio of 1 :20. The concentration of $P_2O_5$ was measured spectrophotometrically (Shimadzy UV 2600, Japan), while the concentration of $K_2O$ was measured by flame photometrically (Jenway 6105, USA). The concentration of available Fe, Mn, and Zn micronutrients in the soil was determined after the extraction with a buffered DTPA solution (soil : solution ratio 1 : 2, using an Atomic absorption spectrophotometer (Shimadzu 6300) [ISO 14870:2001].

The assessment of micronutrient contents in leaves and apple fruit dry matter was done by wet digestion with a mixture of nitric acid ($HNO_3$) and hydrochloric acid (HCl) in 1 : 3 ratio. The contents of the total Fe, Mn, and Zn were determinate by AAS method (Shimadzu 6300).

Statistical analysis was performed using the *t*-test to compare the two seasons, and the analysis of variance (ANOVA) procedures and the means were separated by the Tukey's multiple range test at $P \leq 0.05$ and 0.01.

Based on the content of the microelements in the fruits, the partial nutrient balance (PNB) of the microelement was calculated and it shows the ratio between the quantity of the nutrient removed by the yield and the amount applied by the fertilizers [Fixen et al. 2015]. Partial nutrient balance on the plot is calculated according to the formula:

$$\text{PNB (\%)} = \frac{\text{Removed amount of microelement (g ha}^{-1}\text{)}}{\text{Applied amount of microelement through fertilizers (g ha}^{-1}\text{)}} \times 100$$

## RESULTS AND DISCUSSION

### Fe content

The application of both doses of N fertilizers had a positive impact on the Fe content in leaves in both years (Tabs 1, 2), although the measured Fe concentration in the soil as well as in the leaves was satisfactory even in control treatments [Hanson 1996]. The positive influence of N on the adsorption of Fe is explained by various physiological and molecular mechanisms that are directly dependent on the plant provision with N [Cakmak et al. 2010]. In 2014, the largest impact on Fe content in leaves was observed after a moderate application of N and by using foliar fertilization with salts. The influence of N on Fe absorption and its content in leaves was conditioned by the synergetic interaction between these two elements in the plant tissue [Jivan and Sala 2014]. The highest Fe content in apple fruits was measured after foliar fertilization with salts and also after fertigation with chelates. In their research, Borowski and Michalek [2011] compared the efficiency of foliar application of non-organic and organic salts of Fe. They found that both types of fertilizers had a significant effect on the increase of the Fe content of in leaves of beans compared to control; however, the foliar application of the non-organic form Fe $(NO_3)_2$ showed the greatest, while the Fe-EDTA the smallest influence.

In both year of experiment, a significantly higher concentration, compared to control, of Fe in apple leaves was measured at all treatments with micronutrients application except on treatment where Fe sulphate was applied trough fertigation (Tabs 1 and 2). The absence of the effect of this treatment can be explained by well-known facts that the microelements in the chelated form in relation to the inorganic forms (salts of sulphate, chloride, etc.) are more resistant to the oxidation and transformation processes that reduces the Fe availability, especially in the alkaline soil [Morgan and Lahav 2007]

### Mn content

Under the influence of fertilizers, Mn content in leaves and fruits was no different due to its high concentration in the soil (24.33 mg Mn $kg^{-1}$), much higher than the critical level of 1 mg Mn $kg^{-1}$ [Lindsay and Norvell 1978]. Depending on the form of Mn fertilizer, the method of application and fertilization with N, the Mn content in the apple leaves ranged from 139 to 176 and 69 to132 mg Mn $kg^{-1}$, in the first and the second year, respectively. In all treatments, measured values were higher than 50 mg Mn $kg^{-1}$, which was reported as the lowest limit for optimal plant provision [Hoying et al. 2004]. However, in the first year the concentration of Mn in leaves and fruits was significantly higher than in second (Tab. 3). The differences in mean values of Mn in leaves and fruits between two years were probably a consequence of the great influence of soil moisture on the Mn availability in soil. In the first year we had an extremely high amount of precipitation which could lead to waterlogged, less aerated soil, and reduction of the $Mn^{4+}$ and $Mn^{3+}$ into a plant available $Mn^{2+}$ form [Marschner 1988, Porter 2004].

In the second year (Tab. 3), there was a statistically significant effect of N fertilization and micronutrients on leaves contents. The highest Mn content in leaves was observed after foliar fertilization with Mn salts as well as after fertilization with the highest level of N, which is as in case of Fe, a consequence of their synergism [Chaplin and Martin 2008]. Previous studies related to the foliar application of Mn indicate a higher application efficiency of Mn in the form of sulphates compared to the application by chelates [Thalheimer and Paoli 2002, Papadakis et al. 2005]. However, fertigation with Mn-sulphate did not affect the Mn content in leaves.

### Zn content

In both years of research fertilization with nitrogen and foliar application of Zn sulphate had a positive effect on the content of Zn in the leaf and apple fruit (Tabs 1 and 2). On other hand, the application of Zn fertilizers through fertigation did not have an effect on the content of Zn in the leaf and fruit in the first year of research, while in the second year, only the application of Zn in the form of chelate led to an increase in Zn content in leaves and fruits. In the first year of the research, the application of Zn fertilizers through fertigation did not have an effect on the content of Zn in the leaves and the fruits most likely due to the large amount of precipitation that could lead to the rinsing of Zn fertilizers into deeper soil layers, outside the root system (Graf 1).

**Table 1.** Effects of fertilization with N and microelements on the content of Fe, Mn and Zn in the leaves and fruits and on the number of fruits per tree, average fruit weight and total yield in 2014

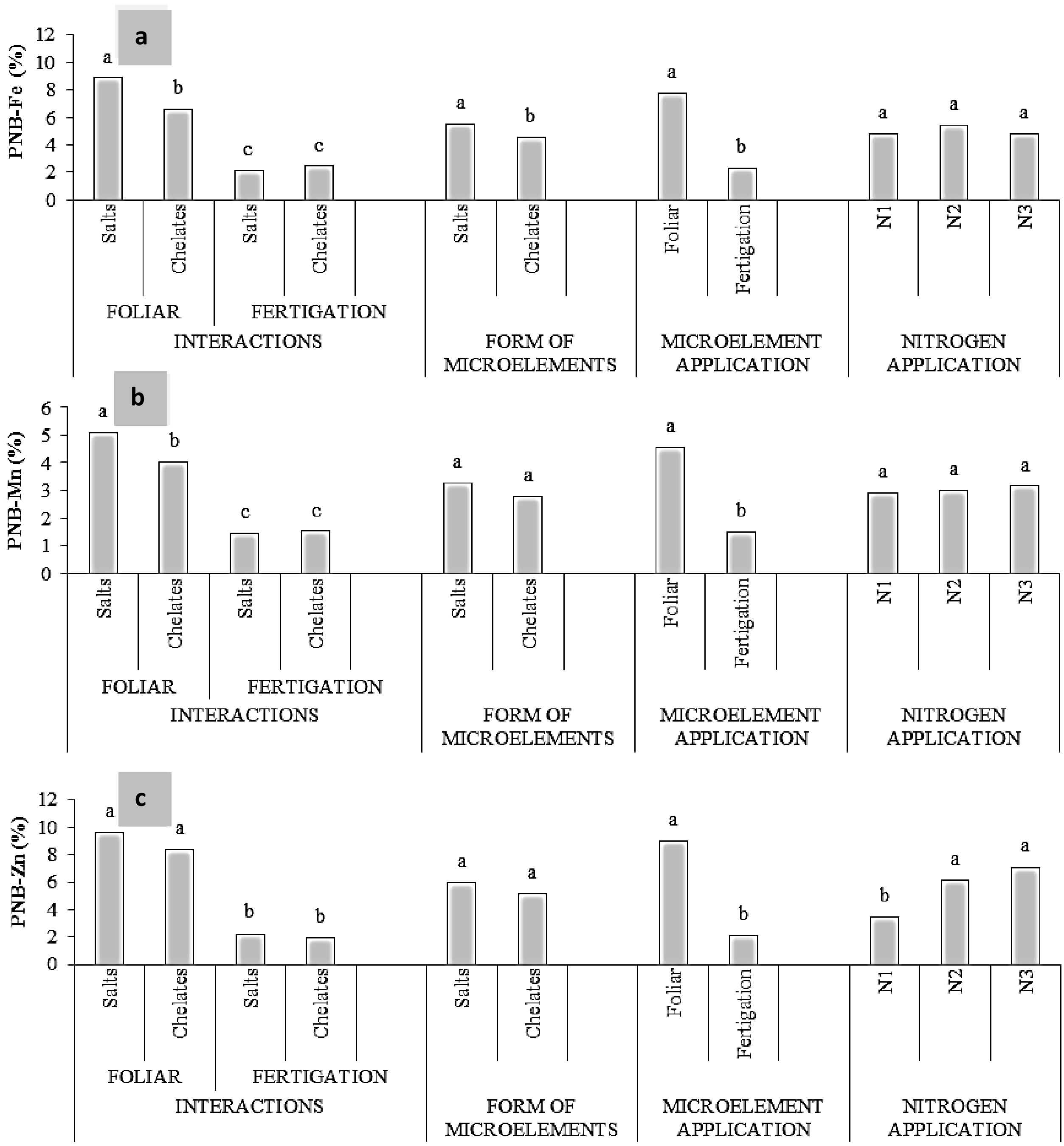
| Treatments | | Leaf | | | Fruit | | | Fruit | | |
|---|---|---|---|---|---|---|---|---|---|---|
| N | M | Fe | Mn | Zn | Fe | Mn | Zn | weight | number | yield |
| | | mg kg$^{-1}$ | | | | | | g fruit$^{-1}$ | | kg tree$^{-1}$ |
| $N_0$ | 1 | $148_{efg}$ | $139_a$ | $23_b$ | $12_{cd}$ | $3.2_a$ | $2.3_e$ | $224_b$ | $32_a$ | $7.22_a$ |
| | 2 | $183_{bcde}$ | $156_a$ | $26_b$ | $18_{bcd}$ | $3.1_a$ | $4.2_{cde}$ | $234_b$ | $34_a$ | $8.06_a$ |
| | 3 | $187_{bcd}$ | $161_a$ | $28_b$ | $20_{ab}$ | $3.8_a$ | $4.1_{cde}$ | $231_b$ | $34_a$ | $7.79_a$ |
| | 4 | $137_{fg}$ | $142_a$ | $23_b$ | $12_{cd}$ | $3.2_a$ | $2.3_e$ | $216_b$ | $32_a$ | $6.96_a$ |
| | 5 | $148_{efg}$ | $162_a$ | $25_b$ | $11_d$ | $3.8_a$ | $3.8_{cde}$ | $237_{ab}$ | $31_a$ | $7.36_a$ |
| | 6 | $148_{efg}$ | $161_a$ | $30_b$ | $13_{cd}$ | $3.1_a$ | $4.2_{cde}$ | $229_b$ | $34_a$ | $7.84_a$ |
| $N_{80}$ | 1 | $169_{cdef}$ | $154_a$ | $27_b$ | $14_{bcd}$ | $2.9_a$ | $5.9_{abcd}$ | $246_{ab}$ | $35_a$ | $8.70_a$ |
| | 2 | $199_{bc}$ | $165_a$ | $29_b$ | $14_{bcd}$ | $3.0_a$ | $7.0_{abc}$ | $252_{ab}$ | $33_a$ | $8.27_a$ |
| | 3 | $283_a$ | $176_a$ | $43_a$ | $24_a$ | $3.9_a$ | $8.0_{ab}$ | $256_{ab}$ | $31_a$ | $8.02_a$ |
| | 4 | $124_g$ | $159_a$ | $27_b$ | $12_{cd}$ | $2.9_a$ | $5.9_{abcd}$ | $238_{ab}$ | $35_a$ | $8.42_a$ |
| | 5 | $199_{bc}$ | $164_a$ | $26_b$ | $18_{abc}$ | $3.2_a$ | $5.0_{bcde}$ | $220_b$ | $35_a$ | $7.71_a$ |
| | 6 | $136_{fg}$ | $152_a$ | $22_b$ | $16_{bcd}$ | $3.4_a$ | $3.6_{cde}$ | $235_b$ | $34_a$ | $8.05_a$ |
| $N_{160}$ | 1 | $155_{defg}$ | $161_a$ | $21_b$ | $13_{cd}$ | $3.3_a$ | $3.1_{de}$ | $263_a$ | $35_a$ | $9.32_a$ |
| | 2 | $215_b$ | $157_a$ | $47_a$ | $13_{cd}$ | $3.0_a$ | $7.7_{ab}$ | $258_a$ | $32_a$ | $8.24_a$ |
| | 3 | $187_{bcd}$ | $173_a$ | $47_a$ | $16_{bcd}$ | $3.6_a$ | $8.0_{ab}$ | $264_a$ | $35_a$ | $9.14_a$ |
| | 4 | $122_g$ | $161_a$ | $21_b$ | $13_{cd}$ | $3.3_a$ | $3.1_{de}$ | $263_a$ | $35_a$ | $9.32_a$ |
| | 5 | $155_{defg}$ | $157_a$ | $22_b$ | $18_{abc}$ | $3.2_a$ | $3.5_{de}$ | $265_a$ | $37_a$ | $9.93_a$ |
| | 6 | $146_{efg}$ | $161_a$ | $27_b$ | $13_{cd}$ | $3.4_a$ | $6.0_{abcd}$ | $266_a$ | $32_a$ | $8.56_a$ |
| | 1 | $157_d$ | $151_b$ | $24_c$ | $9_b$ | $3.1_b$ | $3.8_{bc}$ | $244_a$ | $34_a$ | $8.4_a$ |
| | 2 | $199_b$ | $159_{ab}$ | $34_{ab}$ | $15_b$ | $3.0_b$ | $6.3_{ab}$ | $248_a$ | $33_a$ | $8.2_a$ |
| | 3 | $219_a$ | $170_a$ | $39_a$ | $20_a$ | $3.8_a$ | $6.7_a$ | $250_a$ | $33_a$ | $8.3_a$ |
| | 4 | $128_d$ | $154_b$ | $24_c$ | $12_b$ | $3.1_b$ | $3.8_{bc}$ | $239_a$ | $34_a$ | $8.2_a$ |
| | 5 | $167_c$ | $161_{ab}$ | $24_c$ | $16_b$ | $3.4_{ab}$ | $5.1_b$ | $241_a$ | $34_a$ | $8.3_a$ |
| | 6 | $143_d$ | $158_{ab}$ | $26_{bc}$ | $14_b$ | $3.3_b$ | $4.6_{bc}$ | $243_a$ | $33_a$ | $8.2_a$ |
| $N_0$ | | $158{,}5_b$ | $154_a$ | $26_b$ | $13_b$ | $3{,}4_a$ | $3{,}5_b$ | $229_b$ | $33_a$ | $7{,}5_b$ |
| $N_{80}$ | | $185{,}0_a$ | $168_a$ | $29_{ab}$ | $16_a$ | $3{,}2_a$ | $5{,}9_a$ | $241_b$ | $34_a$ | $8{,}2_{ab}$ |
| $N_{160}$ | | $163{,}3_{aba}$ | $167_a$ | $31_a$ | $14_b$ | $3{,}3_a$ | $5{,}7_a$ | $263_a$ | $34_a$ | $9{,}1_a$ |

N – treatments: 1. control; 2. 80 kg N ha$^{-1}$; 3. 160 kg N ha$^{-1}$;
M – microelement treatment: 1. foliar control; 2. foliar fertilization with chelates; 3. foliar fertilization with salts; 4. fertigation control; 5. fertigation with chelates; 6. fertigation with salts.
Small letters represent a difference at 95% between data in the column

**Table 2.** Effects of fertilization with N and microelements on the content of Fe, Mn and Zn in the leaves and fruits and on the number of fruits per tree, average fruit weight and total yield in 2015

| Treatments | | Leaf | | | Fruit | | | Fruit | | |
|---|---|---|---|---|---|---|---|---|---|---|
| N | M | Fe | Mn | Zn | Fe | Mn | Zn | weight | number | yield |
| | | mg $kg^{-1}$ | | | | | | g $fruit^{-1}$ | | kg $tree^{-1}$ |
| $N_0$ | 1 | $151.9_{d}$ | $83_{bc}$ | $32_{g}$ | $22_{d}$ | $2.4_{a}$ | $2.6_{ef}$ | $181_{a}$ | $55_{c}$ | $9.87_{bc}$ |
| | 2 | $191.1_{bcd}$ | $96_{abc}$ | $36_{efg}$ | $40_{abc}$ | $2.6_{a}$ | $4.1_{bcdef}$ | $177_{a}$ | $52_{c}$ | $9.12_{c}$ |
| | 3 | $217.6_{abc}$ | $103_{abc}$ | $46_{abcdef}$ | $41_{abc}$ | $2.7_{a}$ | $4.6_{abcdef}$ | $187_{a}$ | $59_{abc}$ | $11.09_{abc}$ |
| | 4 | $152.5_{d}$ | $69_{c}$ | $32_{g}$ | $22_{d}$ | 2.3a | $2.6_{f}$ | $184_{a}$ | $55_{c}$ | $10.05_{bc}$ |
| | 5 | $241.0_{ab}$ | $92_{bc}$ | $56_{ab}$ | $43_{abc}$ | $2.6_{a}$ | $6.0_{abcd}$ | $196_{a}$ | $55_{abc}$ | $10.80_{abc}$ |
| | 6 | $194.5_{bcd}$ | $83_{bc}$ | $34_{fg}$ | $31_{bcd}$ | $2.5_{a}$ | $3.9_{cdef}$ | $185_{a}$ | $49_{c}$ | $9.00_{c}$ |
| $N_{80}$ | 1 | $187.5_{bcd}$ | $84_{bc}$ | $42_{defg}$ | $30_{bcd}$ | $2.1_{a}$ | $3.6_{def}$ | $175_{a}$ | $61_{abc}$ | $10.61_{abc}$ |
| | 2 | $214.4_{abc}$ | $100_{abc}$ | $43_{cdefg}$ | $36_{abcd}$ | $2.4_{a}$ | $4.6_{abcdef}$ | $185_{a}$ | $53_{c}$ | $9.74_{bc}$ |
| | 3 | $244.2_{ab}$ | $120_{ab}$ | $54_{abcd}$ | $50_{a}$ | $3.0_{a}$ | $6.9_{ab}$ | $180_{a}$ | $68_{abc}$ | $12.13_{abc}$ |
| | 4 | $187.1_{bcd}$ | $89_{bc}$ | $43_{cdefg}$ | $30_{bcd}$ | $2.1_{a}$ | $4.9_{abcdef}$ | $180_{a}$ | $61_{abc}$ | $10.91_{abc}$ |
| | 5 | $256.0_{a}$ | $103_{abc}$ | $55_{abc}$ | $43_{abc}$ | $2.7_{a}$ | $7.4_{a}$ | $194_{a}$ | $72_{abc}$ | $13.91_{ab}$ |
| | 6 | $174.5_{cd}$ | $100_{abc}$ | $46_{abcdef}$ | $28_{cd}$ | $2.1_{a}$ | $4.1_{bcdef}$ | $186_{a}$ | $67_{abc}$ | $12.47_{abc}$ |
| $N_{160}$ | 1 | $178.3_{cd}$ | $93_{bc}$ | $44_{bcdefg}$ | $30_{bcd}$ | $2.5_{a}$ | $4.0_{cdef}$ | $169_{a}$ | $63_{abc}$ | $10.70_{abc}$ |
| | 2 | $230.7_{abc}$ | $107_{ab}$ | $49_{abcde}$ | $37_{abcd}$ | $2.7_{a}$ | $4.5_{abcdef}$ | $185_{a}$ | $71_{abc}$ | $13.15_{abc}$ |
| | 3 | $256.0_{a}$ | $132_{a}$ | $54_{abcd}$ | $41_{abc}$ | $3.1_{a}$ | $5.5_{abcde}$ | $180_{a}$ | $79_{ab}$ | $14.11_{ab}$ |
| | 4 | $178.1_{cd}$ | $92_{bc}$ | $36_{efg}$ | $30_{bcd}$ | $1.8_{a}$ | $4.0_{cdef}$ | $169_{a}$ | $63_{abc}$ | $10.70_{abc}$ |
| | 5 | $261.1_{a}$ | $108_{ab}$ | $57_{a}$ | $37_{abcd}$ | $3.0_{a}$ | $6.6_{abc}$ | $186_{a}$ | $82_{a}$ | $15.26_{a}$ |
| | 6 | $218.1_{abc}$ | $101_{abc}$ | $46_{abcdef}$ | $29_{bcd}$ | $3.0_{a}$ | $5.3_{abcdef}$ | $172_{a}$ | $63_{abc}$ | $10.77_{abc}$ |
| | 1 | $173_{c}$ | $87_{b}$ | $39_{c}$ | $27_{c}$ | $2.3_{c}$ | $3.4_{c}$ | $175_{b}$ | $60_{a}$ | $10.4_{b}$ |
| | 2 | $212_{ab}$ | $101_{ab}$ | $43_{c}$ | $38_{b}$ | $2.6_{bc}$ | $4.4_{bc}$ | $182_{ab}$ | $59_{a}$ | $10.7_{b}$ |
| | 3 | $239_{a}$ | $118_{a}$ | $51_{ab}$ | $44_{a}$ | $2.9_{a}$ | $5.7_{ab}$ | $182_{ab}$ | $69_{a}$ | $12.4_{ab}$ |
| | 4 | $173_{c}$ | $83_{b}$ | $37_{c}$ | $27_{c}$ | $2.1_{c}$ | $3.8_{c}$ | $178_{b}$ | $60_{a}$ | $10.6_{b}$ |
| | 5 | $253_{a}$ | $101_{ab}$ | $56_{a}$ | $41_{ab}$ | $2.8_{ab}$ | $6.7_{a}$ | $192_{a}$ | $70_{a}$ | $13.3_{a}$ |
| | 6 | $196_{bc}$ | $95_{ab}$ | $42_{c}$ | $29_{c}$ | $2.6_{bc}$ | $4.4_{bc}$ | $181_{ab}$ | $60_{a}$ | $10.7_{b}$ |
| $N_0$ | | $192_{b}$ | $88_{b}$ | $39_{b}$ | $33_{a}$ | $2.5_{a}$ | $4.0_{b}$ | $185_{a}$ | $54_{b}$ | $10.0_{b}$ |
| $N_{80}$ | | $211_{ab}$ | $99_{ab}$ | $47_{a}$ | $36_{a}$ | $2.4_{a}$ | $5.3_{a}$ | $183_{ab}$ | $64_{ab}$ | $11.6_{ab}$ |
| $N_{160}$ | | $220_{a}$ | $106_{a}$ | $48_{a}$ | $34_{a}$ | $2.7_{a}$ | $5.0_{a}$ | $177_{b}$ | $70_{a}$ | $12.4_{a}$ |

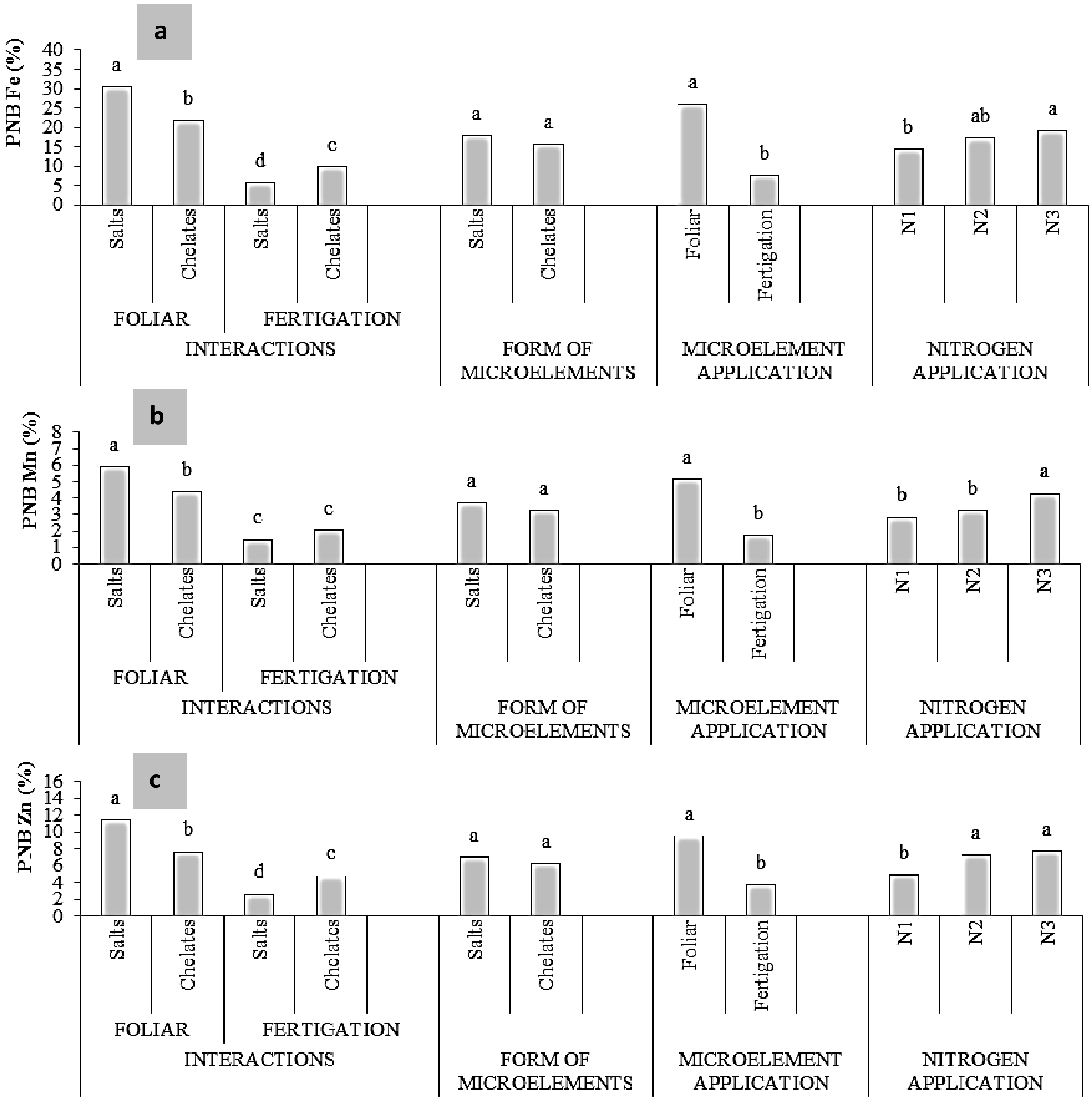
N – treatments: 1. control; 2. 80 kg N $ha^{-1}$; 3. 160 kg N $ha^{-1}$
M – microelement treatment: 1. foliar control; 2. foliar fertilization with chelates; 3. foliar fertilization with salts; 4. fertigation control; 5. fertigation with chelates; 6. fertigation with salts
Small letters represent a difference at 95% between data in the column




N – nitrogen application: 1. control; 2. 80 kg N ha$^{-1}$; 3. 160 kg N ha$^{-1}$

The values marked with different letters are statistically significantly different ($p < 0.05$)

**Fig. 2.** Partial nutrient balance (PNB) in 2014 for: a) Fe; b) Mn; c) Zn




N – nitrogen application: 1. control; 2. 80 kg N $ha^{-1}$; 3. 160 kg N $ha^{-1}$.
The values marked with different letters are statistically significantly different ($p < 0.05$)

**Fig. 3.** Partial nutrient balance (PNB) in 2015 for: a) Fe; b) Mn; c) Zn

In both years of research, the highest content of Zn in the leaves and fruits of apples was measured on the treatment with foliar application of zinc sulphate. These results are in line with previous research of Modaihsh [1997] and Doolette et al. [2018] who reported a higher efficiency of foliar application of Zn sulfate in relation to Zn helate, but opposite to results of Patel et al. [1995] and Shivay et al. [2014] who reported higher efficacy of foliar application of Zn if it is used in the form of Zn chelate.

**Number of fruits per tree, average fruit weight and total yield**

The application of N fertilizers had a positive influence on the total yield in both years of experiment (Tabs 2 and 3). The application of N fertilizers in the first year led to an increase in total yield through an increase in the average weight of the fruit, without affecting the total number of fruits per tree in the second year nitrogen fertilization led to an increase in yield due to the significantly larger number of fruits per tree compared to control, but at the same time significantly lower average fruit.

In the first year, the application of N fertilizers did not lead to an increase in the total number of fruits, since the process of differentiation of buds, from which depends the number of fruits per tree, has already been completed in the spring 2014.

Such results are fully in line with Wargo et al. [2003] and Milić et al. [2012], who reported a positive impact of N fertilizer application on the total number of fruits in apples, and the negative correlation between fruit number per tree and average fruit weight. On the other hand, the application of microelements did not affect the overall yield of apples, except in the second year of the study for the treatment with the microelements applied by fertigation in form of chelates (Tab. 2).

**Partial nutrient balance**

In our study, through foliar application of microelements, we added three times less active substance than with the application by fertigation. In order to evaluate the efficiency of different methods and the form of microelements application, a partial nutrient balance was calculated as one of the indicators of the efficiency of fertilizer application [Fixen et al. 2015]. This factor represents the ratio between the amount of microelements removed by yield in relation to the total amount applied and it is expressed as percentage. Snyder and Bruulsema [2007] state that the ideal values for PNB should be 1, or 100%, which would mean that the nutrient removed was the same as that amount applied of. Values higher than 100% would mean that removed amount of nutrient from the plot is higher than the return by fertilizers and can come from internal reserves or other external sources, while values significantly below 100% indicate low fertilizer efficiency and potential pollution of the ecosystem. In our study, the foliar application of all three microelements had significantly higher PNB values compared to soil application in both years of research (Fig. 2A and 2B). However, if we exclude the values of PNB for Fe applications in 2015 which ranged between 5% and 34%, the value for Zn and Mn fertilization were below

**Table 3.** Differences in the values of Fe, Mn, Zn, yield, weight and number of fruits per tree in two seasons

| Season | Fe | | Mn | | Zn | | Weight | Number | Yield |
|---|---|---|---|---|---|---|---|---|---|
| Year | leaf | fruit | leaf | fruit | leaf | fruit | fruit | | |
| | mg kg$^{-1}$ | | | | | | g | | kg tree$^{-1}$ |
| 2014 | 174.1 | 15.13 | 158.9 | 3.32 | 28.56 | 4.93 | 244.3 | 33.67 | 8.27 |
| 2015 | 207.5 | 34.75 | 97.5 | 2.53 | 44.67 | 4.74 | 181.9 | 62.67 | 11.36 |
| t-test | ** | ** | ** | ** | ** | NS | ** | ** | ** |

NS – not significant;*significance at the 95%; **significance at the 99%

10% which indicates the relatively low efficiency of the applied fertilizers with microelements.

## CONCLUSIONS

Based on the obtained results, it can be concluded that application of N fertilizers in 80 kg N ha$^{-1}$ nad 160 kg N ha$^{-1}$ led to the accumulation of micronutrients in apple leaves and fruits. In addition to that, the use of N fertilizers had a positive impact on the total yield in both years of research. The highest level of N had the impact on average fruit weight in first year, and total number of fruits per apple tree in second year of research. The strongest effects on the accumulation of micronutrients in leaves were observed using foliar fertilization with salts and fertigation with chelates. The foliar application of Mn, Zn and Fe had significantly higher partial nutrient balance values compared to soil application in both years of the experiment. However, most of the PNB values were below 10% indicating the relatively low efficiency of the applied fertilizers with microelements. Also, the results showed that the effectiveness of fertilizer application with microelements depends on the agroecological conditions during vegetation, where their impact is greater if fertilizers are applied by fertigation, regardless of the form of the microelements.

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