

1 **An investigation of the content of**
2 **manganese in the fruit of marrows (*Cucurbita***
3 ***pepo* L. var. *giromontia*) in respect of the kind**
4 **of fertilizer and the type of fertilization**

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10 **ABSTRACT**

11 **The main aim of this study is to determine the influence of manganese and fertilization upon the nutritious value of fruit of marrows and to clarify the opportunity for using Blue tetrazolium chloride [1] as a reagent for determination of micro quantities of manganese in plant samples.**

The investigation was conducted in the period 2007–2009 in an open field condition with the marrows (*Cucurbita pepo* L. var. *giromontia*) cultivar Izobilna F₁ on the experimental field of the Agricultural University of Plovdiv, Bulgaria.

Manganese is determined with a new developed method with Blue tetrazolium chloride as a chromogenic reagent. The BTC method can be successfully applied to the determination of manganese in plant samples. It is found out that as a result of the investigation of the influence of the kind of fertilizer in foliar and mixed fertilization in marrows (*Cucurbita pepo* L. var. *giromontia*) the content of manganese in the fruit of marrows is highest in foliar fertilization and mixed fertilization with Hortigrow. There has been investigated the subordination between the content of manganese, total carbohydrates and nitrate nitrogen in the fruit of marrows in foliar fertilization and mixed fertilization. Yield decreases with an increase of the amount of accumulated Mn in the fruit of marrows. This subordination can be seen in the different ways of fertilization.

Blue tetrazolium chloride [1] could be used as a reagent for determination of micro quantities of manganese in plant samples.

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15 *giromontia*

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17 1. INTRODUCTION

18 The main objective of scientific investigations in vegetable crop production is to
19 increase plant productivity and obtaining quality yields. One of way to achieve this purpose is
20 to optimize technologies for growing plants. Foliar application is considered as part of the
21 agronomic techniques carried out during the period of cultivation as an opportunity to
22 increase plant productivity [2]; [3]; [4] [5]; [6] [7]. Despite the significant number of
23 investigation on vegetables requirements of foliar fertilization, little is known of content of Mn
24 in vegetables and its connection with foliar fertilization. Information in Bulgarian and foreign
25 scientific literature is scanty. In previous our publications there are data about the influence
26 of foliar fertilizers upon the quality of fruit of marrows [8], as well as about the content of Mo
27 in marrows [9].

28 The determination of manganese essentiality in plant growth focused the attention of
29 plant nutritionists on this nutrient, and led the way for further studies. Manganese has a
30 catalytic role in plants, and combinations with proteins are essential to higher plant life.
31 Manganese is involved in many biochemical functions, primarily acting as an activator of
32 enzymes such as dehydrogenases, transferases, hydroxylases involved in respiration,
33 amino acid and lignin synthesis [10] [11]. Manganese is involved in oxidation-reduction
34 reactions within the photosynthetic electron transport system in plants. Manganese is also
35 involved in the photosynthetic evolution of O₂ in chloroplasts [12]; [13].

36 Manganese is an element which is important for the nitrogen metabolism in plants.
37 Manganese insufficiency leads to a considerable accumulation of nitrates, disturbance in the
38 protein synthesis in plants and illness to some plants [14], [15], [16]. The optimal content of
39 manganese, its critical level and toxic concentration at which the growth is depressed and
40 the yield decreased, have been established for a great number of crops [17]. Also the
41 manganese insufficiency causes a decrease in Ca and Mg contents in plants [18].

42 In the works of [19], [20], [21], [22]; [23], [24] some questions connected with the
43 influence of Mn upon different biochemical processes as well as upon growth of plants are
44 considered.

45 The objective of this study is to determine the manganese content in the fruit of
46 marrows in foliar fertilization with 0.3%Fitona (7.20%N. 5.20%K₂O,1.5%Ca 0.9%Mg.
47 0.1%Fe. 0.1%B, Cu, Zn, Mn, Mo. Fitotech Ltd., Bulgaria) , 0.3 %Hortigrow (20%N 20%P₂O₅

20%K₂O, 0.06%Fe, 0.02%Zn, 0.01%Mn, 0.01%Cu, 0.02%B, 0.001%Mo and 1% amino acids, Hortiland Ltd., Nederland), 0.3% Humustim (3%N, 1.14%P₂O₅, 7.83%K₂O, 3.92%Ca, 1.1%Mg, Cu, Zn, Mo, Co, B, S. Agrospeis Ltd., Bulgaria), and mixed fertilization (soil and foliar). The main aim of this study is to determine the influence of manganese and fertilization upon the nutritious value of fruit of marrows and to clarify the opportunity for using Blue tetrazolium chloride [1] as a reagent for determination of micro quantities of manganese in plant samples.

2. MATERIAL AND METHODS

The investigation was conducted in the period 2007–2009 in an open field condition with the marrows (*Cucurbita pepo* L. var. *giromontia*) cultivar Izobilna F₁ on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The soil is assigned as Mollic Fluvisols [25]. The power of the humus horizon is 28-30cm. The mechanical structure soil is loam texture. Content of physical clay is from 30% to 41%. Humus content is low - 1.46%, pH - neutral to slightly alkaline (7.17-7.37). The presence of large amounts of CaCO₃ gives more favorable physical-chemical water and soil properties, despite the heavy mechanical composition. Nitrogen, in the soil is poor reserve (32-46mg.kg⁻¹), good stocks with removable phosphorus (P₂O₅ - 16.7-18 mg.kg⁻¹) and many good stocks with removable potassium (K₂O - 67-96 mg.kg⁻¹).

For the purpose of the experiment complete foliar fertilizers Fitona (7.20%N, 5.20%K₂O, 1.5%Ca, 0.9%Mg, 0.1%Fe, 0.1%B, Cu, Zn, Mn, Mo, Fitotech Ltd., Bulgaria) Hortigrow (20%N, 20%P₂O₅, 20%K₂O, 0.06%Fe, 0.02%Zn, 0.01%Mn, 0.01%Cu, 0.02%B, 0.001%Mo and 1% amino acids, Hortiland Ltd., Nederland) and Humustim (on base of potassium humates-3%N, 1.14%P₂O₅, 7.83%K₂O, 3.92%Ca, 1.1%Mg, Cu, Zn, Mo, Mn, Co, B, S. Agrospeis Ltd., Bulgaria), were used. The ratio of soil fertilization was N₁₆P₁₆K₁₆. Phosphorus and potassium fertilizers were applied with last tillage of soil before planting. Nitrogen fertilizer was applied twice during the growing season. First application was after formation of new leaves of plants, and the second - 20 days after the first. Indices: N₁₆ – introduced as NH₄NO₃ (34 % N) ; P₁₆ – introduced as Ca(H₂PO₄)₂ (46 % P₂O₅); K₁₆ – introduced as K₂SO₄ (50 % K₂O).

Water solution of foliar fertilizers was prepared. Foliar fertilizers are brought in the given concentrations three times in the phases- beginning of flowering, beginning of fruit production, mass fruit production. Solution with the needed concentration is prepared for the different variants. Control plants are treated with pure water. The consumption of working solution in the first spraying is 60 l.da⁻¹, and in the second and third 80 l.da⁻¹.

Plants are cultivated according to the conventional technology for early field

production of marrows, as previously produced seedlings are used [26]. The seedling is on thirty days, cultivated in non-heated polythene hothouse. Plants are planted on bed-furrow surface, according to scheme 100+60/50 cm and density of plantation 2500 number of plants per da in beginning the May. Growth period is 45 days after planting.

Total carbohydrates were determined by Haygedorn – Jensen [27] and dray matter-weight method [27]. Manganese was determined in the fruits of marrows [1]. Materials were collected in mass fruit production

Variants of the experiment: 1. Control - non fertilized ; 2. Foliar fertilization with 0.3 % Fitona; 3. Foliar fertilization with 0.3 %Hortigrow; 4. Foliar fertilization with 0.3 % Humustim; 5. Soil fertilization with $N_{16}P_{16}K_{16}$; 6. Soil fertilization with $N_{16}P_{16}K_{16}$ + 0.3 %Fitona; 7. Soil fertilization with $N_{16}P_{16}K_{16}$ + 0.3 %Hortigrow ; 8. Soil fertilization with $N_{16}P_{16}K_{16}$ +0.3 %Humustim;

Determination of manganese (VII) with BTC in plant samples

An analysis for manganese content by variants was carried out using a new extraction-spectrophotometric method with Blue tetrazolium chloride [1]. Manganese (VII) forms an ion-association complex BTC. The molar absorptivity of the ion-association complex is $4.97 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 255 nm. Beer's law is obeyed in the range of 0.1 – 1.6 $\mu\text{g ml}^{-1}$. Limit of deterction (DL) is $6.86 \text{ ng ml}^{-1} \text{ Mn(VII)}$ and limit of quantitation (QL) is $22.8 \text{ ng ml}^{-1} \text{ Mn(VII)}$. The determination was carried out without preliminary separation of manganese. 1g from the plant material should be reduced to ashes in an oven in $450 - 500^\circ \text{C}$. The dry residium should be dissolved in a dilute hydrochloric acid (1:1). It was transferred into a volumetric flask of 50 ml and diluted to the mark with distilled water. Aliquot parts of this solution were taken for analysis. An aliquot of a sample solution was transferred into 100 ml separatory funnel. Add 2 ml of Blue tetrazolium chloride $1 \times 10^{-4} \text{ mol l}^{-1}$, 0.5 ml of sulphuric acid 2 mol l^{-1} . The resulting solution was diluted with distilled water to a total volume of 10 ml. Then 3 ml of 1,2-dichloroethane was added, and the funnels were shaken for 15 s. Transfer the organic layer through a dry filter paper into a 1 cm cuvette and measure the absorbance at 255 nm against a reagent blank similarly prepared [1].

Statistical analysis

Relative Standard Deviation about Mn determined according to the BTC method is figured out. The obtained results about total carbohydrates and dry matter are mathematically processed using the dispersion analysis method for one factor field trial [28], as the program product BIOSTAT (ANOVA) was used.

Apparatus

Spectrophotometer UV- VIS with 1-cm light path quartz cells; Flame Atomic Absorbtion Spectrophotometer “Perkin Elmer”(Germany);

3. RESULTS AND DISCUSSION

The content of microelement manganese in the fruit of marrows is represented on Table 1 in foliar fertilization with Fitona 0.3%, Hortigrow 0.3 %, Humustim 0.3 % and mixed fertilization (soil and foliar). The experimental data show that the content of manganese in the fruit of marrows is highest in fertilization with Hortigrow (foliar fertilization and mixed fertilization). This is due to the fact that in the composition of the foliar fertilizer Hortigrow can be found 0.01 % Mn. Similarly Hortigrow comprises aminoacids that stimulate growth, development and assimilation of nutritious substances including microelements. The distinction in the content of manganese in the two variants of fertilization is 0.40 mg.kg⁻¹. Hence, the additional introduction of N, P and K in soil has small influence on the accumulation of manganese in the fruit of marrows.

In the same variants of fertilization the content of total carbohydrates in the fruit of marrows has been determined. The content of total carbohydrates is highest in soil and foliar fertilization with N₁₆P₁₆K₁₆ + Hortigrow. The experimental data (Table 1) show that the content of total carbohydrates is higher 2.39 % in mixed fertilization. In foliar fertilization the content of total carbohydrates is 0.78 % or three times less than that in mixed fertilization. The content of total carbohydrates in the control (non fertilized) is less 0.67 %. The content of carbohydrates is higher when we use the foliar fertilizer Hortigrow together with the soil fertilization (N₁₆P₁₆K₁₆) that was observed when we use manganese. The content of dry matter in fruit in the different kinds of fertilization is represented on Table 3. The dry matter in the fruit of marrows is almost the same in fertilization with Hortigrow and Humustim (foliar and mixed fertilization). An exception can be discerned in fertilization with the foliar fertilizer Fitona, in which the difference in the dry substance is 2.6 % between foliar and mixed fertilization.

TABLE 1. Content of manganese, total carbohydrates and dry matter in the fruit of marrows in foliar and mixed fertilization

№	Variants	Mn, mg kg ⁻¹ BTC method	*RSD Mn	** Total carbohydrates%	*** Dry matter, %
1	Control - non fertilized	9.67	1.9	0.67	6.02
2	0.3 % Fitona	8.32	1.5	0.79	6.97
3	0.3 % Hortigrow	9.70	1.2	0.78	6.18
4	0.3 % Humustim	8.69	1.7	0.92	6.45
5	N ₁₆ P ₁₆ K ₁₆	8.48	2.0	1.82	4.80
6	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Fitona	8.63	1.4	1.34	4.41
7	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Hortigrow	10.10	1.9	2.39	5.94
8	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Humustim	8.85	1.7	1.42	5.70

* Relative Standard Deviation for BTC method (n = 5)

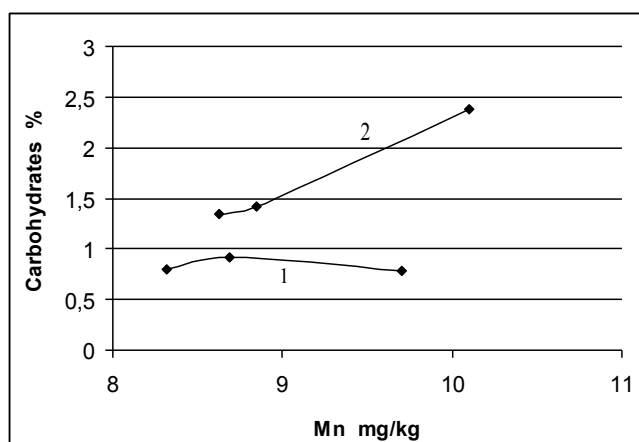
** Total carbohydrates % - LSD 0.949, P=0.05

*** Dry matter, % - LSD 0.782, P=0.05

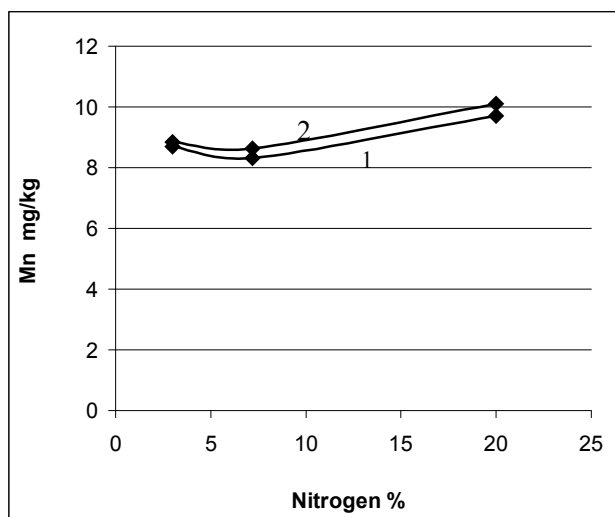
148 In a recent review, [7] state that the foliar feeding up is due to the ability of leaves
 149 and stems to absorb the nutritious elements taken in them in the form of certain solutions.
 150 The effect of the foliar feeding-up takes place much promptly in comparison to the soil
 151 fertilization. It is also very important to mention that the physiological diseases of plants
 152 caused by deficiency or scarcity of a given element can be avoided. [6].

153 The relationship between the content of manganese and total carbohydrates in the
 154 fruit of marrows is represented on Fig. 1. In foliar fertilization (Fig.1, curve 1) the content of
 155 total carbohydrates varies in the interval 0.78 % - 0.92 % and is lower than that in mixed
 156 fertilization. The experimental data show that in foliar fertilization in the highest content of
 157 Mn 9.70 mg.kg^{-1} in fruits, there could be found the least amount of carbohydrates 0.78 %.
 158 The highest content of total carbohydrates 0.92 % can be found in the content of Mn 8.69
 159 mg.kg^{-1} in the fruit of marrows. It makes an impression that in foliar fertilization with
 160 Hortigrow the content of carbohydrates is three times lower in comparison with mixed
 161 fertilization with this foliar fertilizer (soil fertilization + 0.3 % Hortigrow).

162 In mixed fertilization (Fig.1, curve 2) a direct subordination can be seen between the
 163 accumulated Mn in the fruit of plants and the content of carbohydrates. The content of total
 164 carbohydrates in the fruit increases with the increase of the concentration of manganese in
 165 the above mentioned plants. The content of total carbohydrates varies in the interval 1.34 %
 166 - 2.39 % in mixed fertilization. The experimental data show that in the highest content of Mn
 167 10.10 mg.kg^{-1} the content of carbohydrates in fruit is also high 2.39 %. Content of
 168 carbohydrates is several times higher in mixed fertilization in comparison to foliar fertilization.
 169 This all indicates that the nutritious elements N, P and K that has been brought up
 170 additionally on leafs have positive influence on the accumulation of carbohydrates in the fruit
 171 of marrows.



174 **Fig. 1** Subordination between the content of Mn in the fruit of marrows and
 175 carbohydrates: 1- Foliar fertilization; 2- Soil and foliar fertilization
 176 In order to trace out what is the influence of the different fertilization foliar and mixed
 177 one, we have investigated the influence of nitrogen that is being incorporated in different
 178 fertilizers upon the content of manganese in the fruit of marrows. The experimental data (Fig.
 179 2) show that together with the increase of the percent content of nitrogen in fertilizer, the
 180 content of Mn increases to 9.7 mg.kg^{-1} for 20 % nitrogen in foliar fertilizer, and respectively
 181 10.1 mg.kg^{-1} Mn for 20 % nitrogen in mixed fertilizer.
 182 Consequently the higher content of nitrogen in fertilizer helps for the accumulation of
 183 bigger amount of manganese in the fruit of plants.



184 **Fig. 2.** Subordination between the content of Mn in the fruit of marrows and nitrogen in foliar
 185 fertilizer; Curve 1: foliar fertilization, Curve 2: soil and foliar fertilization
 186 The subordination between the content of nitrate nitrogen and the content of
 187 manganese in the fruit of marrows is represented on Fig.3. In foliar fertilization with the
 188 increase of the content of nitrate nitrogen from 90 mg.kg^{-1} to 250 mg.kg^{-1} the content of Mn
 189 increases from 8.32 mg.kg^{-1} to 9.7 mg.kg^{-1} in the fruit.
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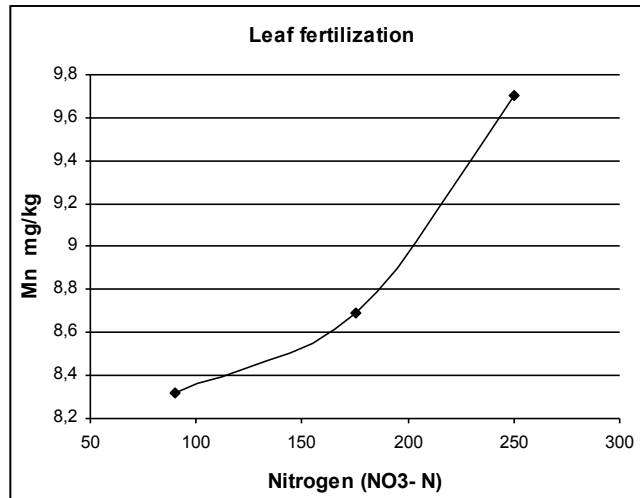


Fig. 3 Subordination between the content of nitrate nitrogen ($\text{NO}_3 - \text{N}$) and Mn in the fruit of marrows in foliar fertilization

In foliar and soil fertilization (Fig. 4) the highest content of Mn 10.1 mg.kg^{-1} responds to the least amount of nitrate nitrogen 100 mg.kg^{-1} that is being assimilated by plants. The experiments show that with the increase of nitrate nitrogen, the amount of the accumulated manganese in fruit decreases.

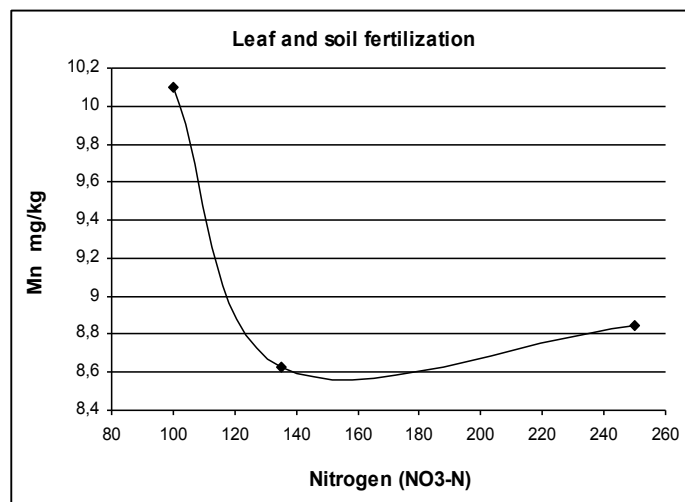


Fig. 4 Subordination between the content of nitrate nitrogen ($\text{NO}_3 - \text{N}$) and Mn in the fruit of marrows in foliar and soil fertilization

Manganese is an important nutritious element for plants that has an influence on the quality of yield and production. In this respect the influence of manganese upon the yield of marrows in the two variants of fertilization (foliar fertilization and mixed fertilization) is represented on Fig. 5 and Fig.6. The researches showed that in the control (non fertilized),

the content of manganese is lower as in foliar fertilization and also in mixed fertilization. In independent foliar fertilization the highest yield 3917 $\text{kg}\cdot\text{da}^{-1}$ is obtained in the content of Mn 8.69 $\text{mg}\cdot\text{kg}^{-1}$ in the fruit of marrows (Fig. 5). In mixed fertilization the highest yield 4945 $\text{kg}\cdot\text{da}^{-1}$ is obtained in the content of Mn 8.85 $\text{mg}\cdot\text{kg}^{-1}$ in the fruit of marrows (Fig. 6). The experimental data show that in mixed fertilization, yield is with 1000 $\text{kg}\cdot\text{da}^{-1}$ higher than that in foliar fertilization. It makes an impression that the content of manganese is almost equal for the two ways of fertilization in which the highest yield is obtained. Consequently the main factor for higher yield is the different fertilization and not the content of manganese.

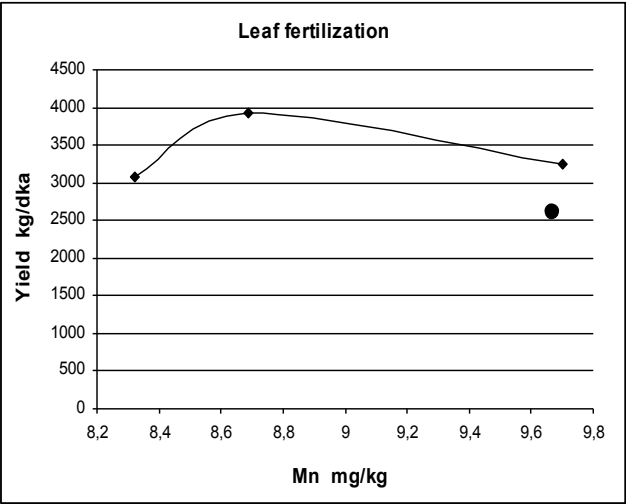


Fig. 5 Subordination between yield and the content of Mn in the fruit of marrows in foliar fertilization; ● – Control (non fertilized)

The influence of the different kind of fertilization upon the yield of fruit is presented on Fig. 7. The experimental data show that mixed fertilization (soil and foliar) leads to the increase of yield up to 1000 $\text{kg}\cdot\text{da}^{-1}$ in comparison with foliar fertilization. The highest yield is obtained in fertilization with Humustim (mixed fertilization and foliar fertilization). This may be due to the fact that in the composition of the organic foliar fertilizer Humustim the basic nutritious elements are humic acids that exhibit hormone activity which stimulates growth and development.

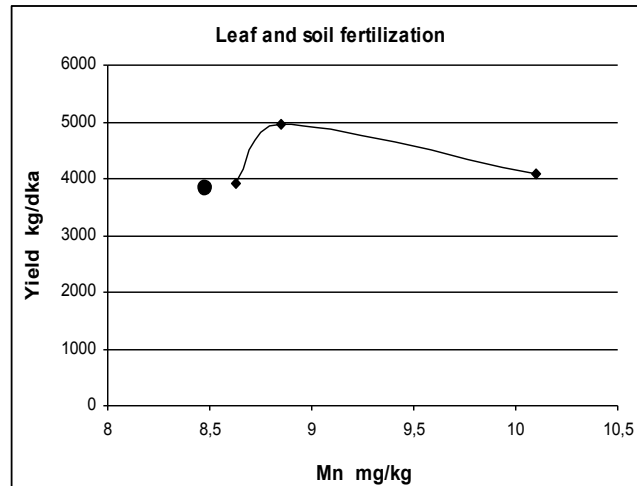


Fig. 6 A subordination between yield and the content of Mn in the fruit of marrows in mixed fertilization (soil and foliar); • – Control (non fertilized)

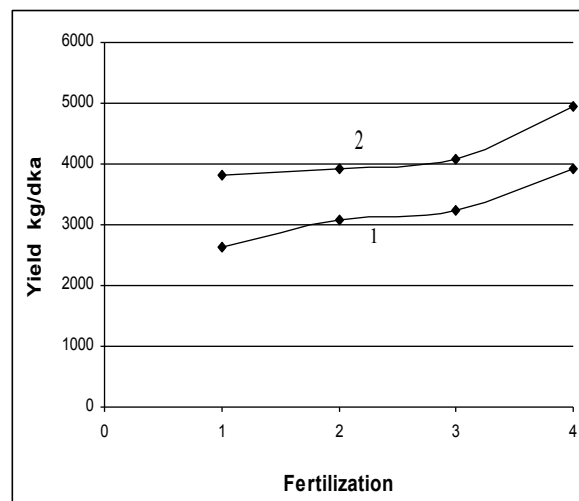


Fig. 7. Subordination between yield from the fruit of marrows and fertilization:
 Curve 1 (foliar fertilization): 1- Control; 2- Fitona; 3- Hortigrow; 4-Humustim
 Curve 2 (mixed fertilization): 1- $N_{16}P_{16}K_{16}$; 2- $N_{16}P_{16}K_{16}$ + Fitona ; 3-
 $N_{16}P_{16}K_{16}$ + Hortigrow ; 4- $N_{16}P_{16}K_{16}$ + Humustim

4. CONCLUSION

The use of foliar fertilizers containing different macro- and micronutrients in the period of growing marrows influenced on the content of Mn in their fruit. The highest content of Mn in the fruits of zucchini was found after combined soil and foliar fertilization with 0.3% Hortigrow

Yield decreases with an increase of the amount of accumulated Mn in the fruit of marrows. The content of total carbohydrates in the fruit increases with the increase of the concentration of manganese in the above mentioned plants. The increase of nitrate nitrogen, the amount of the accumulated manganese in fruit decreases.

Blue tetrazolium chloride (Kostova and Mehandjiev, 2013) could be used as a reagent for determination of micro quantities of manganese in plant samples.

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