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ABSTRACT

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.

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

Keywords: blue tetrazolium chloride, fertilization, manganese, cucurbita pepo L. var. giromontia

1. INTRODUCTION

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

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

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

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

The objective of this study ~~was~~ is to determine the manganese content in the fruit of
marrows ~~after in foliar fertilization with different fertilizers 0.3%Fitona (7.20%N.~~

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5.20%K₂O, 1.5%Ca 0.9%Mg, 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) and . The main aim of this study is to determine the influence of manganese and fertilization upon the nutritious value of fruit of marrows. Furthermore, we aimed at and to clarifying the opportunity possibility 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 under open field conditions with the marrows (*Cucurbita pepo* L. var. *giromontia*) cultivar Izobilna F₁ on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The soil of the field is classified as Molic Fluvisols [25]. The depth power of the humus horizon is 28-30cm. The mechanical structure soil is loamy (clay content is from 30% to 41%). Chemically, the soil is characterized by a Humus content is low content in organic matter (1.46%), pH - neutral to slightly alkaline (7.17-7.37) and by the presence of large amounts of CaCO₃, which gives more favorable physical-chemical water and soil properties, despite the heavy mechanical composition. Nitrogen content was low in the soil is poor reserve (32-46mg.kg⁻¹), while there was a good stocks of soluble 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 three different complete foliar fertilizers were used:

a) 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)

b) 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

a)c) 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.

Soil fertilization was carried out with NPK using a The ratio of soil fertilization was N₁₆P₁₆K₁₆. Phosphorus (Ca(H₂PO₄)₂ - 46% P₂O₅) and potassium (K₂SO₄ - 50% K₂O) fertilizers were applied with last tillage of soil before planting. Nitrogen fertilizer, introduced as NH₄NO₃ (34% N), 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

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NH_4NO_3 (34 % N); P_{16} — introduced as $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (46 % P_2O_5); K_{16} — introduced as K_2SO_4 (50 % K_2O);

Water solution of foliar fertilizers was prepared. Foliar fertilizers were applied ~~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 was prepared for the different ~~variants~~~~treatments~~ treatments. Control plants were ~~are~~ treated with pure water. The consumption of working solution in the first spraying is 600 l.ha^{-1} , and in the second and third 800 l.ha^{-1} .

Plants ~~are~~ cultivated according to the conventional technology for early field production of marrows, ~~as~~ using previously produced seedlings ~~are used~~ [26]. The seedling was planted after ~~is on~~ thirty days, ~~cultivation ed~~ in non-heated polythene ~~hothouse~~ tunnel. Plants ~~we~~ are planted on bed-furrow surface, according to scheme 100+60/50 cm and density of plantation 25000 ~~number of~~ plants per ~~hda~~ in beginning the May. Growth period ~~was~~ 45 days after planting.

Total carbohydrates were determined using the method of ~~by~~ Haygedorn – Jensen [27]. Plant ~~and~~ dry matter was determined according to the ~~weight~~ method of Stanchev et al. [27]. Manganese was determined in the fruits of marrows [1]. Materials were collected in the phase of mass fruit production

~~Variants~~ ~~Treatments~~ 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 $\text{N}_{16}\text{P}_{16}\text{K}_{16}$; 6. Soil fertilization with $\text{N}_{16}\text{P}_{16}\text{K}_{16}$ + 0.3 % Fitona; 7. Soil fertilization with $\text{N}_{16}\text{P}_{16}\text{K}_{16}$ + 0.3 % Hortigrow ; 8. Soil fertilization with $\text{N}_{16}\text{P}_{16}\text{K}_{16}$ + 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 detection (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 was~~ reduced to ashes in an oven in $450 - 500^\circ \text{C}$. The dry ~~residue ium should be was~~ dissolved in a dilute hydrochloric acid (1:1) ~~solution~~. 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

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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 measured at 255 nm against a reagent blank using a Spectrophotometer UV-VIS (Germany) [1]. The obtained results are compared with those of the AAS method. ~~similarly prepared~~ using a Flame Atomic Absorption Spectrophotometer "Perkin Elmer"(Germany); ~~H1~~

Statistical analysis

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

Apparatus

~~Spectrophotometer UV-VIS with 1 cm light path quartz cells; Flame Atomic Absorption Spectrophotometer "Perkin Elmer"(Germany);~~

3. RESULTS AND DISCUSSION

The content of microelement manganese in the fruit of marrows ~~after fertilization~~ with foliar (Fitona 0.3%, Hortigrow 0.3 %, Humustim 0.3 %) and mixed fertilization (soil and foliar) 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 ~~after 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 treatments~~ 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 treatments~~ of fertilization the content of total carbohydrates in the fruit of marrows has been determined. The content of total carbohydrates is ~~the~~ highest in ~~plants~~ receiving soil and foliar fertilization ~~with (N₁₆P₁₆K₁₆ + Hortigrow)~~. The experimental data (Table 1) show that the content of total carbohydrates is ~~the~~ highest (2.39 %) ~~after in~~ mixed fertilization. ~~In~~ After only 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 ~~even lower~~ (0.67-%). The content of carbohydrates is higher when we used the foliar fertilizer Hortigrow together with the soil fertilization (N₁₆P₁₆K₁₆), a result that paralleled that was observed for when we use the manganese content in fruits.

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The content of dry matter in fruit in the different kinds of fertilization is represented on Table 13. The dry matter in the fruit of marrows is almost the same after in-fertilization with Fitona, Hortigrow, and (Hortigrow + soil fertilization), Humustim, (Humustim + soil fertilization) and control, (foliar and mixed fertilization). An exception can be discerned in fertilization with (Fitona + soil fertilization) and soil fertilization $N_{16}P_{16}K_{16}$ 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

No	Variants	Mn, mg kg ⁻¹ BTC method	*RSD BTC method Mn	** Total carbohydrates %	*** Dry matter , %	AAS Mn mg kg ⁻¹
1	Control - non fertilized	9.67	1.9	0.67	6.02	9.80
2	0.3 % Fitona	8.32	1.5	0.79	6.97	8.00
3	0.3 % Hortigrow	9.70	1.2	0.78	6.18	9.55
4	0.3 % Humustim	8.69	1.7	0.92	6.45	8.80
5	$N_{16}P_{16}K_{16}$	8.48	2.0	1.82	4.80	8.65
6	$N_{16}P_{16}K_{16}$ + 0.3 % Fitona	8.63	1.4	1.34	4.41	8.55
7	$N_{16}P_{16}K_{16}$ + 0.3 % Hortigrow	10.10	1.9	2.39	5.94	10.30
8	$N_{16}P_{16}K_{16}$ + 0.3 % Humustim	8.85	1.7	1.42	5.70	8.67

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

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

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

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

The relationship between the content of manganese and total carbohydrates in the fruit of marrows is represented on Fig. 1. In With foliar fertilization (Fig.1, curve 1) the content of total carbohydrates varies in the interval 0.78 % - 0.92 % and is lower than that in mixed fertilization (curve 2). The experimental data show that in f There was no correlation between the amount of Mn in leaves and the amount of carbohydrates adte foliar fertilization in the highest content of Mn 9.70 mg.kg⁻¹ in fruits, there could be found the least amount of carbohydrates 0.78 %. The highest content of total carbohydrates 0.92 % can be found in the content of Mn 8.69 mg.kg⁻¹ in the fruit of marrows. On NN the other hand, mixed fertilization (Fig.1, curve 2) showed a direct correlation between the accumulated Mn in the fruit of plants and the content of carbohydrates. It makes an impression that in foliar

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fertilization with Hortigrow the content of carbohydrates is three times lower in comparison with mixed fertilization with this foliar fertilizer (soil fertilization + 0.3 % Hortigrow).

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

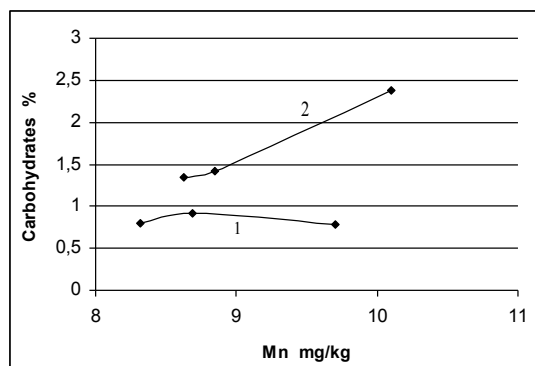


Fig. 1 Subordination Correlation between the content of Mn in the fruit of marrows and the content of carbohydrates: 1- Foliar fertilization; 2- Soil and foliar fertilization

In order to characterize trace-out what is the influence of the different fertilization foliar and mixed fertilizations one, we have investigated the influence of nitrogen that was applied is being incorporated in by the different fertilizers upon the content of manganese in the fruit of marrows. The experimental data (Fig. 2) show that together with the increase of the percent content of nitrogen in fertilizer, the content of Mn increases to 9.7 mg.kg^{-1} for 20 % nitrogen in foliar fertilizer, and respectively to 10.1 mg.kg^{-1} Mn for 20 % nitrogen in mixed fertilizer. We conclude that Consequently the higher content of nitrogen in fertilizers helps for the accumulation of bigger amount of manganese in the fruit of plants.

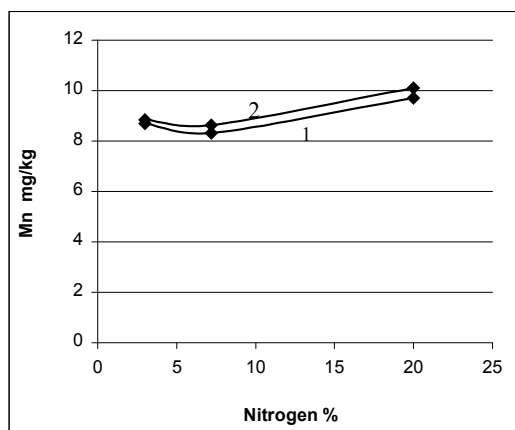
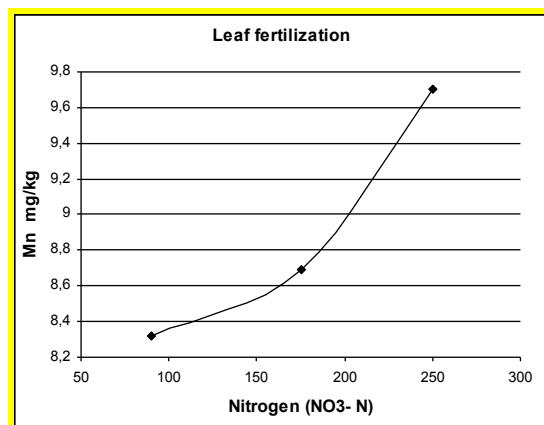


Fig. 2. Subordination Correlation between the content of Mn in the fruit of marrows and nitrogen in foliar fertilizer; Curve 1: foliar fertilization, Curve 2: soil and foliar fertilization

The subordination relationship between the content of nitrate nitrogen and the content of manganese in the fruit of marrows is represented on Fig.3 and 4. The relationship is strikingly opposite for the kinds of fertilization. In With foliar fertilization with the increase of the content of nitrate nitrogen from 90 mg.kg^{-1} to 250 mg.kg^{-1} is paralleled by an increase of the content of Mn increases from 8.32 mg.kg^{-1} to 9.7 mg.kg^{-1} in the fruit. On the other hand, the increase of nitrate nitrogen provided by mixed (soil and foliar) fertilization is correlated to a decrease of Mn in the furits: 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. May be in mixed fertilization (soil and foliar) the introduced in soil macroelements cause influence which can be given to the different content of manganese.



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

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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.

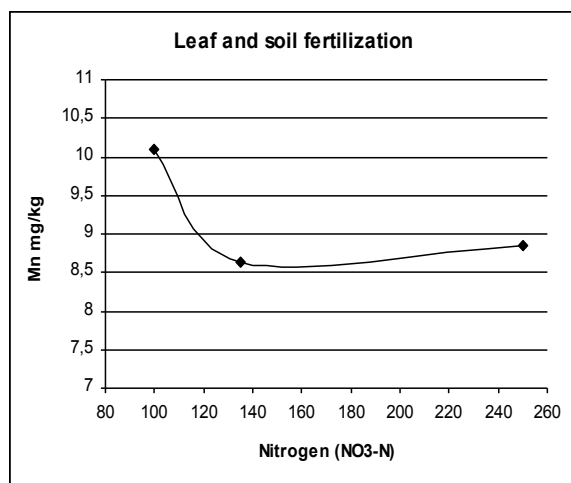
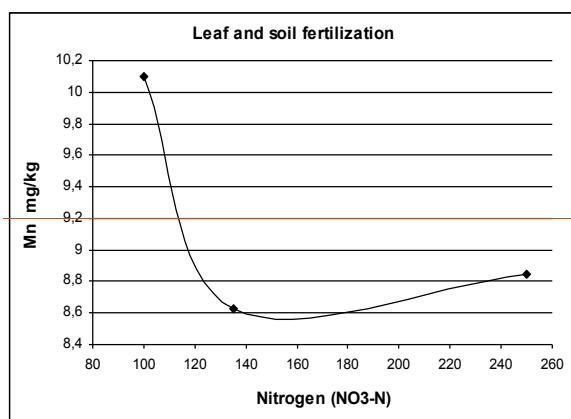


Fig. 4 Subordination between the content of nitrate nitrogen ($\text{NO}_3 - \text{N}$) and Mn in the fruit of marrows in foliar and soil fertilization

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

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marrows in the two variants kinds of fertilization (foliar fertilization and mixed fertilization) is represented on Table 2 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 39170 kg.hda⁻¹ is obtained in the content of Mn 8.69 mg.kg⁻¹ in the fruit of marrows (Fig. 5). In mixed fertilization the highest yield 4945 kg.da⁻¹ is obtained in the content of Mn 8.85 mg.kg⁻¹ in the fruit of marrows (Fig. 6). The experimental data show that in mixed fertilization, yield is with 10000 kg.ha⁻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.

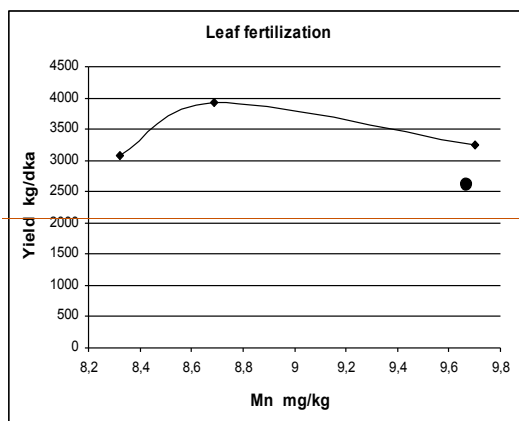


Table 2 Subordination between yield and the content of Mn in the fruit of marrows in foliar fertilization and in mixed fertilization (soil and foliar);

No	Variants	Mn, mg kg ⁻¹ BTC method	Yield kg ha ⁻¹
1	Control - non fertilized	9.67	26210
2	0.3 % Fitona	8.32	30840
3	0.3 % Hortigrow	9.70	32430
4	0.3 % Humustim	8.69	39170
5	N ₁₆ P ₁₆ K ₁₆	8.48	38270
6	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Fitona	8.63	39270
7	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Hortigrow	10.10	40840
8	N ₁₆ P ₁₆ K ₁₆ + 0.3 % Humustim	8.85	49450

Yield kg ha⁻¹- LSD 113.522 P=0.05

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. 57. The experimental data show that mixed fertilization (soil and foliar) leads to the increase of yield up to 10000 kg.hda^{-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.

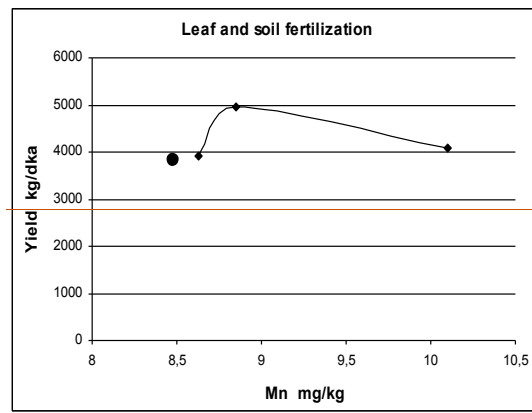


Fig. 6 A subordination between yield and the content of Mn in the fruit of marrows in mixed fertilization (soil and foliar); ● — Control (non-fertilized)

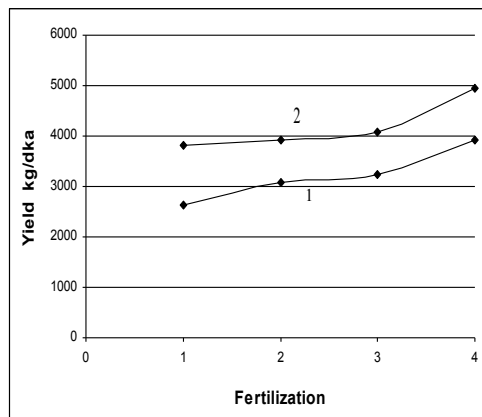


Fig. 57. Subordination between yield from the fruit of marrows and fertilization:
Curve 1 (foliar fertilization): 1- Control; 2- Fitona; 3- Hortigrow; 4-Humustim
Curve 2 (mixed fertilization): 1- $N_{16}P_{16}K_{16}$; 2- $N_{16}P_{16}K_{16}$ + Fitona ; 3-
 $N_{16}P_{16}K_{16}$ + Hortigrow ; 4- $N_{16}P_{16}K_{16}$ + 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

The decrease in yield induces a concentration in manganese. Yield decreases with an increase of the amount of accumulated Mn in the fruit of marrows. The content of total carbohydrates in the fruit of marrows increases with the increase of the concentration of manganese in the above mentioned plants. The increasing the amount of nitrate nitrogen in fertilizers induces a decrease in the amount of the accumulated manganese in fruits decreases. The trend is more clearly expressed in the mixed application.

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

ACKNOWLEDGEMENTS

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372