

1 An investigation of the content of
2 manganese in the fruit of marrows ~~(*Cucurbita*~~
3 ~~*pepo* L. var. *giromontia*)~~, yield and nutritious
4 value of fruit in respect of the kind of fertilizer
5 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, foliar
12 and soil fertilization
 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 F,
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 (foliar plus soil 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.

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

1. INTRODUCTION

Optimization of technologies for growing plants is a method to increase plant productivity and to obtain quality yields. 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 deficiency leads to a considerable accumulation of nitrates, disturbance in the protein synthesis in plants and 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 to determine the manganese content in the fruit of marrows after foliar fertilization with different fertilizers and mixed fertilization (soil and foliar) and to determine the influence of manganese and fertilization upon the nutritious value

of fruit of marrows. Furthermore, we aimed at clarifying the 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 under open field conditions with 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 Mollic Fluvisols [25]. The depth of the humus horizon is 28–30 cm. The soil is loamy (clay content from 30% to 41%). Chemically, the soil is characterized by a 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 (32–46 mg.kg⁻¹), while there was a good stock of soluble phosphorus (P₂O₅ – 16.7–18 mg.kg⁻¹) and 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)
- 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).

Soil fertilization was carried out with NPK using a ratio 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.

Water solution of foliar fertilizers was prepared. Foliar fertilizers were applied 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 treatments. Control plants were 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 were cultivated according to the conventional technology for early field production of marrows, using previously produced seedlings [26]. The seedling was planted after thirty days cultivation in non-heated polythene tunnel. Plants were planted on bed-

furrow surface, according to scheme 100+60/50 cm and density of plantation 25000 plants per ha in beginning the May. Growth period was 45 days after planting.

Total carbohydrates were determined using the method of Haygedorn – Jensen [27]. Plant dry matter was determined according to the 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

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 $N_{16}P_{16}K_{16}$; 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 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 4.97×10^4 L mol⁻¹ cm⁻¹ at 255 nm. Beer's law is obeyed in the range of 0.1 – 1.6 µg ml⁻¹. Limit of detection (DL) is 6.86 ng ml⁻¹ Mn(VII) and limit of quantitation (QL) is 22.8 ng ml⁻¹ Mn(VII). The determination was carried out without preliminary separation of manganese. 1g from the plant material was reduced to ashes in an oven in 450 – 500° C. The dry residue 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 1×10^{-4} 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 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. using a Flame Atomic Absorbtion Spectrophotometer "Perkin Elmer"(Germany); .

Statistical analysis

Relative Standard Deviation about Mn was determined according to the BTC method.. The results from the determination of total carbohydrates and dry matter were elaborated using the dispersion analysis method for one factor field trial [28], using the program BIOSTAT (ANOVA).

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 presented on Table 1. . The experimental data show that the content of manganese in the fruit of marrows is highest after 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 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 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 (N₁₆P₁₆K₁₆ + Hortigrow). The experimental data (Table 1) show that the content of total carbohydrates is the highest (2.39 %) after mixed fertilization. After only foliar fertilization, the content of total carbohydrates is 0.78%, or three times less than 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 observed for the manganese content in fruits.

The content of dry matter in fruit in the different kinds of fertilization is represented on Table 1. The dry matter in the fruit of marrows is almost the same after fertilization with Fitona, Hortigrow, (Hortigrow + soil fertilization), Humustim, (Humustim + soil fertilization) and control. An exception can be discerned in fertilization with (Fitona + soil fertilization) and soil fertilization N₁₆P₁₆K₁₆, .

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 BTC method	** 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)

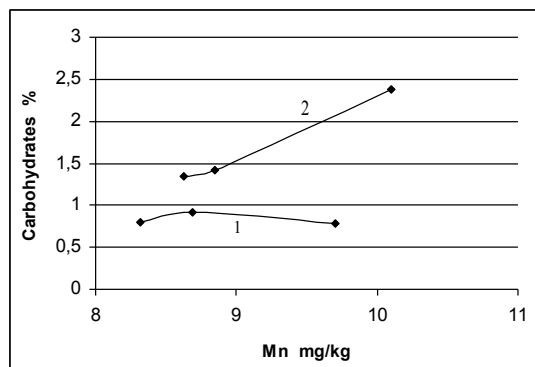
146 ** Total carbohydrates % - LSD 0.949, P=0.05

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

148 In a recent review, [7] it was stated that the foliar uptake is due to the ability of
149 leaves and stems to absorb the nutritious elements taken in in the form of certain solutions.
150 The effect of the foliar nutrition takes place much promptly in comparison to the soil
151 fertilization, which reduces the risks of physiological diseases of plants caused by deficiency
152 or scarcity of a given element [6].

153 The relationship between the content of manganese and total carbohydrates in the
154 fruit of marrows is presented on Fig. 1. With 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 (curve 2). There was no correlation between the amount of Mn in leaves and the
157 amount of carbohydrates adte foliar fertilization. On the other hand, mixed fertilization (Fig.1,
158 curve 2) showed a direct correlation between the accumulated Mn in the fruit of plants and
159 the content of carbohydrates.

160 In. the content of total carbohydrates in the fruit increases with the increase of the
161 concentration of manganesen the highest content of Mn (10.10 mg.kg^{-1}) relates to the
162 highest content of carbohydrates in fruits. Content of carbohydrates is several times higher in
163 mixed fertilization in comparison to foliar fertilization. This all indicates that the nutritious
164 elements N, P and K that has been provided additionally have positive influence on the
165 accumulation of carbohydrates in the fruit of marrows.



166
167
168 Fig. 1 Correlation between the content of Mn in the fruit of marrows and the content
169 of carbohydrates: 1- Foliar fertilization; 2- Soil and foliar fertilization

170
171 In order to characterize the influence of the foliar and mixed fertilizations, we have
172 investigated the influence of nitrogen that was applied by the different fertilizers upon the
173 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. We conclude that the higher content of nitrogen in fertilizers helps for the accumulation of bigger amount of manganese in the fruit of plants.

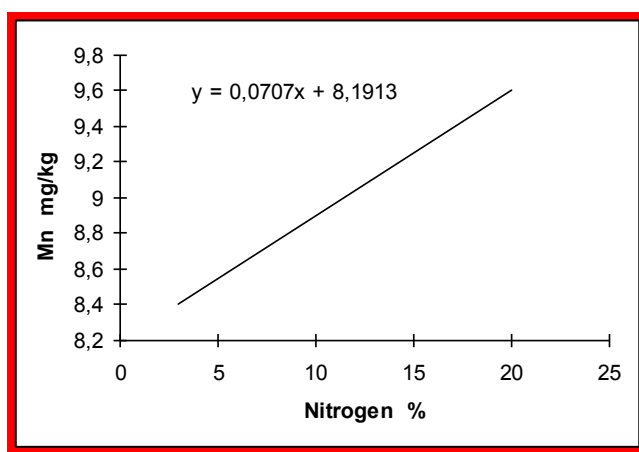


Fig. 2. Correlation between the content of Mn in the fruit of marrows and nitrogen in fertilizers: $r = +0.877$

The 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. With foliar fertilization 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 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 fruits: 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.

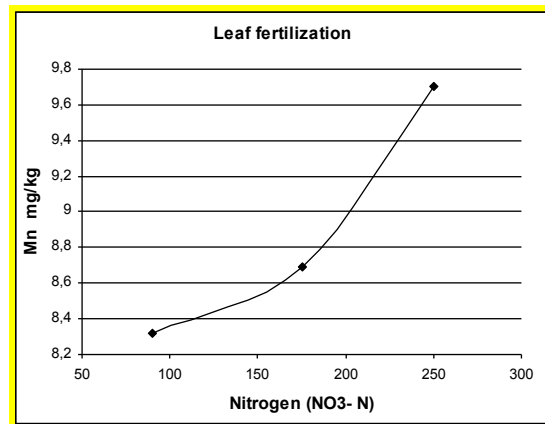


Fig. 3 Correlation between the content of nitrate nitrogen ($\text{NO}_3 - \text{N}$) and Mn in the fruit of marrows in foliar fertilization

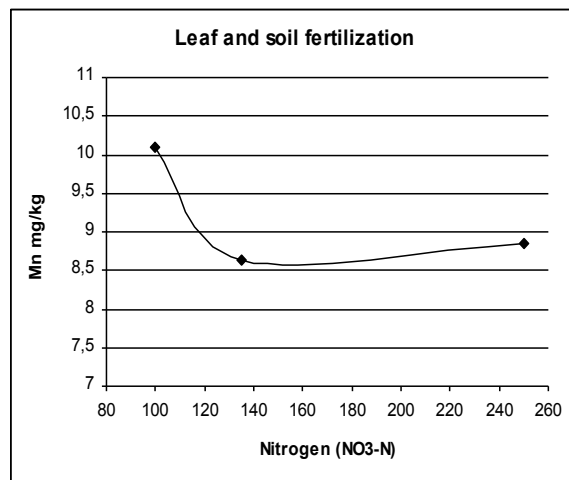


Fig. 4 Subordination between the content of nitrate nitrogen ($\text{NO}_3 - \text{N}$) and Mn in the fruit of marrows in foliar and soil fertilization

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 kinds of fertilization (foliar fertilization and mixed fertilization) is represented on Table 2 . 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 $39170 \text{ kg} \cdot \text{ha}^{-1}$ is obtained in the content of

Mn 8.69 mg.kg⁻¹ in the fruit of marrows. 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. 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

The influence of the different kind of fertilization upon the yield of fruit is presented on Fig. 5. The experimental data show that mixed fertilization (soil and foliar) leads to the increase of yield up to 10000 kg.ha⁻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.

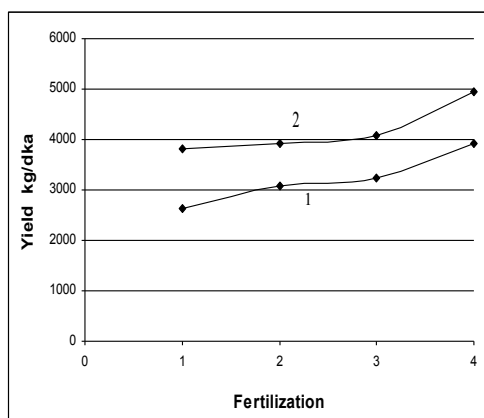


Fig. 5. Subordination between yield from the fruit of marrows and fertilization:
Curve 1 (foliar fertilization): 1- Control; 2- Fitona; 3- Hortigrow; 4-Humustim
Curve 2 (mixed fertilization): 1- $N_{16}P_{16}K_{16}$; 2- $N_{16}P_{16}K_{16}$ + Fitona ; 3-
 $N_{16}P_{16}K_{16}$ + Hortigrow ; 4- $N_{16}P_{16}K_{16}$ + 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 in the fruit of marrows. The content of total carbohydrates in the fruit of marrows increases with the increase of the concentration of manganese. Increasing the amount of nitrate nitrogen in fertilizers induces a decrease in the amount of the accumulated manganese in fruits. The trend is more clearly expressed in the mixed application.

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

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