

Research paper

**AN INVESTIGATION OF THE CONTENT OF MANGANESE IN THE
FRUIT OF MARROWS IN RESPECT OF THE KIND OF FERTILIZER
AND THE TYPE OF FERTILIZATION**

Denka Kostova*, Dimka Haytova*, Dimitar Mehandjiev**

*Agricultural University – Plovdiv, 12 Mendeleev Str., 4000 Plovdiv, Bulgaria
deny_kostova@yahoo.com; haitova@abv.bg

**Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences Acad. G.
Bonchev Str., Bl. 11, 1113 Sofia, Bulgaria
metkomeh@svr.igic.bas.bg

SUMMARY

It is found out that as a result of the investigation of the influence of the kind of fertilizer in leaf and mixed fertilization in marrows the content of manganese in the fruit of marrows is highest in leaf 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.

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.

Keywords: blue tetrazolium chloride, foliar fertilization, manganese, plants

INTRODUCTION

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 (BURNELL, 1988; GRAHAM, 1983). Manganese is involved in oxidation-reduction

reactions within the photosynthetic electron transport system in plants. Manganese is also involved in the photosynthetic evolution of O_2 in chloroplasts (AMESZ, 1993; PRINCE, 1986).

Manganese is an element which is important for the nitrogen metabolism in plants. Manganese insufficiency leads to a considerable accumulation of nitrates, disturbance in the protein synthesis in plants and illness to some plants (NICHOLAS, 1961; BERGMANN and NEUBERT, 1976; HEINTZE, 1966). 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 (POTATUEVA and TARDJIMANIAN, 1990). Also the manganese insufficiency causes a decrease in Ca and Mg contents in plants (SHKOLNIK, 1974).

The objective of this study is to determine the manganese content in the fruit of marrows in foliar fertilization with Fitona 0.3%, Hortigrow 0.3 %, Humustim 0.3 % and mixed fertilization (soil and foliar). To determine the influence of manganese and fertilization upon the nutritious value of fruit of marrows. To clarify the opportunity for using Blue tetrazolium chloride (KOSTOVA and MEHANDJIEV, 2013) as a reagent for determination of micro quantities of manganese in plant samples.

MATERIALS AND METHODS

The investigation was conducted in the period 2007–2009 in an open field condition with the marrows cultivar Izobilna F_1 on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The soil is assigned as Mollic Fluvisols (POPOVA AND SEVOV, 2010). 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 $CaCO_3$ gives more favorable physical-chemical water and soil properties, despite the heavy mechanical composition. Nitrogen, in the soil is poor reserve (32-46mg/kg), good stocks with removable phosphorus (P_2O_5 - 16.7-18 mg/kg) and many good stocks with removable potassium (K_2O - 67-96 mg/kg).

For the purpose of the experiment complete foliar fertilizers (commercial products) Fitona (7.2:0:5.2+M.E.), Hortigrow (20:20:20+M.E.+A.A) and Humustim (on base of potassium humates-3:1.14:7.83+M.E.) were used. The level of soil fertilization was $N_{16}P_{16}K_{16}$.

The thirty days seedlings were grown in an unheated plastic greenhouse. Plants were planted at the beginning of May in density of 2.5 plants per m^2 (100+60/50) The conventional technology to early field production was applied (CHOLAKOV, 2009).

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.

Water solutions of foliar fertilizers were applied three times during vegetation – in the beginning of flowering, beginning of fruitfulness and mass fruitfulness. Control plants sprayed with water. Solution at the first spray was 60 l / da, and in the second and third sprays were 80 l / da.

Variants of the experiment: The experiment was carried out in eight variants: non fertilized, foliar fertilization and mixed fertilization (soil with foliar). 1. Control - non fertilized ; 2. Foliar fertilization 0.3 % Fitona; 3. Foliar fertilization 0.3 % Hortigrow; 4. Foliar fertilization 0.3 % Humustim; 5. Soil fertilization $N_{16}P_{16}K_{16}$; 6. $N_{16}P_{16}K_{16}$ + Fitona 0.3 %; 7. $N_{16}P_{16}K_{16}$ + Hortigrow 0.3 %; 8. $N_{16}P_{16}K_{16}$ + Humustim 0.3%.

Indices: N_{16} – introduced as NH_4NO_3 (34 % N) ; P_{16} – introduced as $Ca(H_2PO_4)_2$ (46 % P_2O_5); K_{16} – introduced as K_2SO_4 (50 % K_2O). The content of nitrogen, phosphorus and potassium in % and the nutritious elements in the used fertilizers is given on Table 1 and Table 2.

An analysis for manganese content by variants were carried out using a new extraction-spectrophotometric method with Blue tetrazolium chloride (KOSTOVA and MEHANDJIEV, 2013). 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.

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

Determination of manganese (VII) with BTC in plant samples: 1g from the plant material should be reduced to ashes in an oven in 450 – 500° C. The dry residuum 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 1×10^{-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 measure the absorbance at 255 nm against a reagent blank similarly prepared (KOSTOVA and MEHANDJIEV, 2013).

RESULTS AND DISCUSSION

The content of microelement manganese in the fruit of marrows is represented on Table 3 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 leaf fertilization with N₁₆P₁₆K₁₆ + Hortigrow. The experimental data (Table 3) 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. As can be seen from Table 3, 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 leaf 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 leaf feeding-up takes place much promptly in comparison to the soil fertilization. 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.

A certain research has been carried out for the content of manganese in the fruit of marrows under the influence of the mineral fertilization ($N_{16}P_{16}K_{16}$) and foliar fertilizers Fitona, Hortigrow, Humustim upon the assimilation of manganese in the fruit of marrows.

The subordination between the content of manganese and total carbohydrates in the fruit of marrows is represented on Fig. 1. In 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. The experimental data show that in 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. It makes an impression that in foliar fertilization with Hortigrow the content of carbohydrates is three times lower in comparison with mixed fertilization with this leaf fertilizer ($N_{16}P_{16}K_{16} + 0.3 \%$ 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} the content of carbohydrates in fruit is also high 2.39 %. From data presented on Fig. 1 it can be seen that the 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 additionally in soil have positive influence on the accumulation of carbohydrates in the fruit of marrows.

In order to trace out what is the influence of the different fertilization foliar and mixed one, we have investigated the influence of nitrogen that is being incorporated in 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 10.1 mg kg^{-1} Mn for 20 % nitrogen in mixed fertilizer. Consequently the higher content of nitrogen in fertilizer helps for the accumulation of bigger amount of manganese in the fruit of plants.

The subordination between the content of nitrate nitrogen and the content of manganese in the fruit of marrows is represented on Fig.3. The experimental data (Fig. 3) show that in leaf

fertilization with the increase of the content of nitrate nitrogen from 90 mg kg⁻¹ to 250 mg kg⁻¹ the content of Mn increases from 8.32 mg kg⁻¹ to 9.7 mg kg⁻¹ in the fruit.

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.

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 kg dka⁻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 dka⁻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 1000 kg dka⁻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.

The content of dry substance in fruit in the different kinds of fertilization is represented on Table 3. The dry substance 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 leaf fertilizer Fitona, in which the difference in the dry substance is 2.6 % between foliar and mixed fertilization.

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 kg dka⁻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.

CONCLUSIONS

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.

The content of total carbohydrates in the fruit increases with the increase of the concentration of manganese in the above mentioned plants. The elements N, P and K that has been brought up additionally in soil have positive influence on the accumulation of carbohydrates in the fruit of marrows.

The increase of nitrate nitrogen, the amount of the accumulated manganese in fruit decreases.

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TABLE 1 .Content of nitrogen, phosphorus and potassium in the fertilizers

Fertilizer	N %	P%	K%
Fitona	7.20	-	5.20
Humustim	3.00	1.14	7.83
Hortigrow	20.00	20.0	20.00

TABLE 2. Content of nutritious elements in the fertilizers

Fertilizer	Content of nutritious elements %
Fitona	Ca – 1.5; Mg- 0.9; Fe-0.1; B-0.1; Cu, Zn, Mn, Mo
Humustim	Ca – 3.92; Mg- 1.1; Cu, Zn, Mo, Co, B, S
Hortigrow	Fe- 0.06; Zn- 0.02; Mn- 0.01; Cu- 0.01; B- 0.02; Mo- 0.001

TABLE 3. 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*	Total carbo- hydrates, %	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)

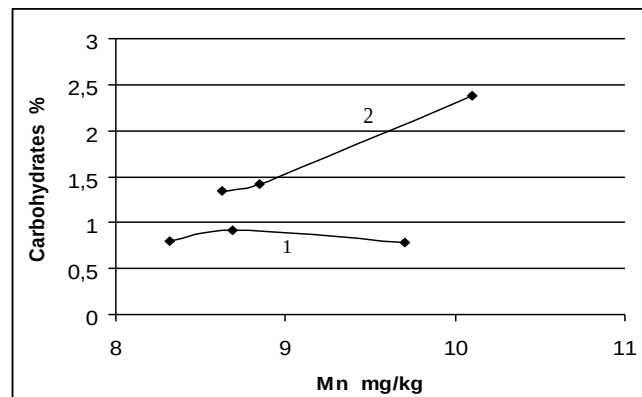


Fig. 1. Subordination between the content of Mn in the fruit of marrows and carbohydrates: 1- Foliar fertilization; 2- Soil and leaf fertilization

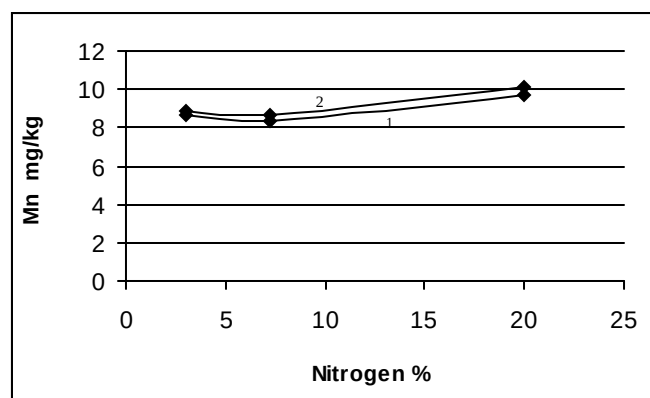


Fig. 2. Subordination between the content of Mn in the fruit of marrows and nitrogen in foliar fertilizer; Curve 1: foliar fertilization, Curve 2: soil and foliar fertilization

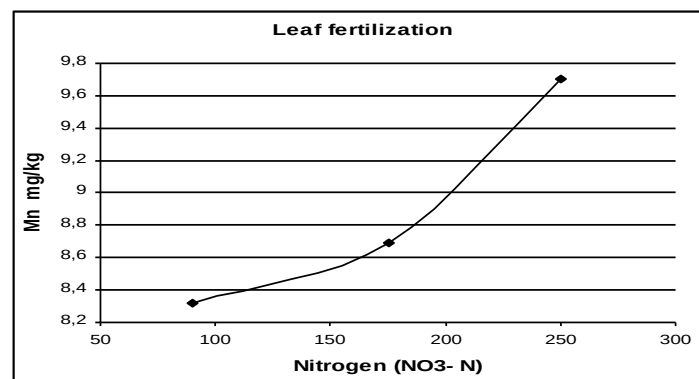


Fig. 3. Subordination between the content of nitrate nitrogen (NO₃ - N) and Mn in the fruit of marrows in leaf fertilization

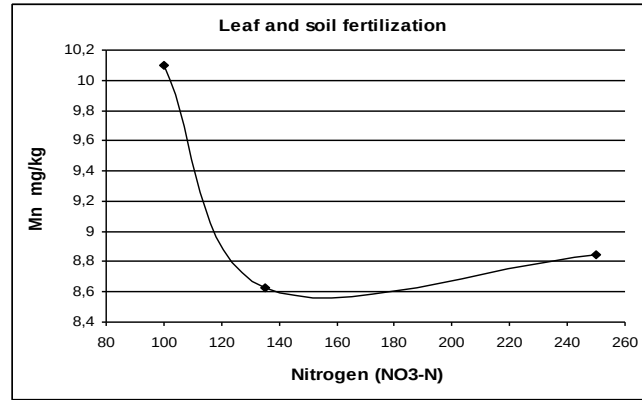


Fig. 4. Subordination between the content of nitrate nitrogen (NO₃ - N) and Mn in the fruit of marrows in leaf and soil fertilization

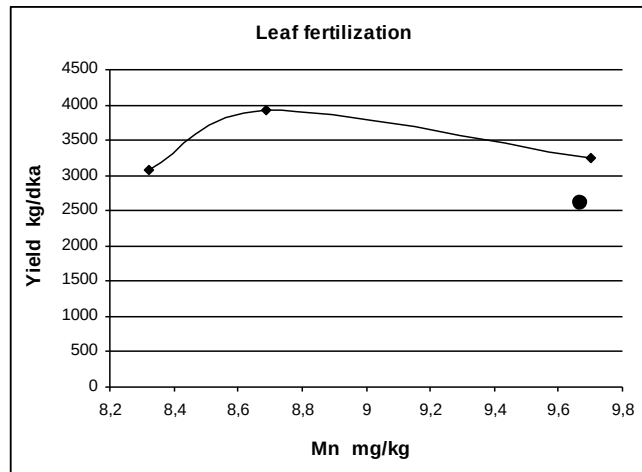


Fig. 5. A subordination between yield and the content of Mn in the fruit of marrows in foliar fertilization; ● – Control (non fertilized)

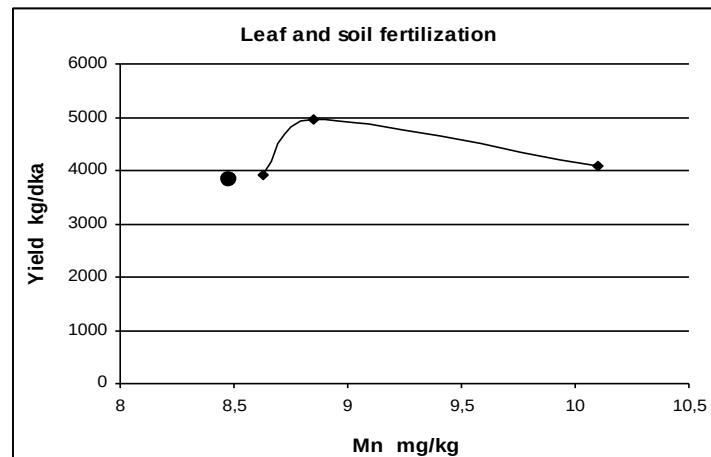


Fig. 6. A subordination between yield and the content of Mn in the fruit of marrows in mixed fertilization (soil and foliar); ● – Control (non fertilized)

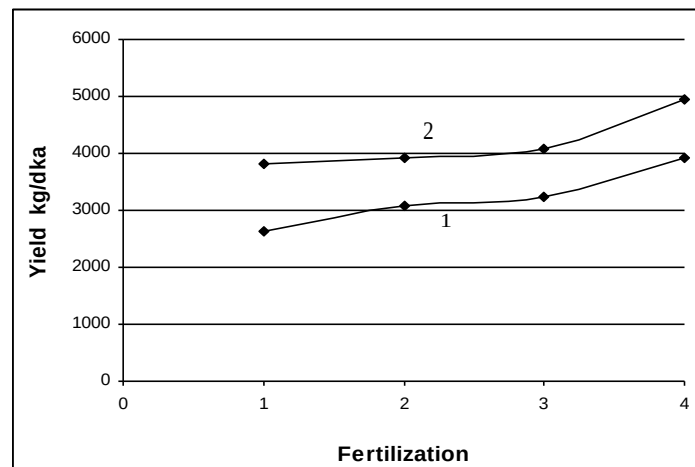


Fig. 7. Subordination between yield from the fruit of marrows and fertilization: *Curve 1 (leaf fertilization)*: 1- Control; 2- Fitona; 3- Hortigrow; 4-Humustim *Curve 2 (mixed fertilization)*: 1- $N_{16}P_{16}K_{16}$; 2- $N_{16}P_{16}K_{16}$ + Fitona ; 3- $N_{16}P_{16}K_{16}$ + Hortigrow ; 4- $N_{16}P_{16}K_{16}$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