

Effect of type and method of fertilization on marrows (*Cucurbita pepo* L.) yield and fruit quality

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ABSTRACT

Aims: The main aim of this study is to determine the influence of foliar and soil fertilization upon the nutritious value of fruit of marrows and to clarify the opportunity for using Blue tetrazolium chloride (BTC) as a reagent for determination of micro quantities of manganese in plant samples.

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

Methodology: 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 N₁₆₀P₁₆₀K₁₆₀; 6. Soil fertilization with N₁₆₀P₁₆₀K₁₆₀ + 0.3 % Fitona; 7. Soil fertilization with N₁₆₀P₁₆₀K₁₆₀ + 0.3 % Hortigrow; 8. Soil fertilization with N₁₆₀P₁₆₀K₁₆₀ + 0.3 % Humustim. Total carbohydrates were determined using the method of Haygedorn—Jensen. Plant dry matter was determined according to the method of Stanchev et al. The content of nitrates of fruits was determined whit test method GOST 29270-95. Mand manganese was determined in the fruits of marrows. Materials were collected in the phase of mass fruit production.

Results: The results show that application of foliar fertilizers Fitona, Hortigrow and Humustim and increased yields of marrows in preserved and improved product quality, the dry matter, total sugars and content of nitrates in fruits. The effect occurred more strongly when it was applied on a background of soil fertilization. The BTC method can be successfully applied to the determination of manganese in plant samples.

Conclusion: The results show that application of foliar fertilizers Fitona, Hortigrow and Humustim and increased yields of marrows in preserved and improved product quality, the dry matter, total sugars and content of nitrates in fruits. The effect occurred more strongly when it was applied on a background of soil fertilization.

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 marrows 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 trend is more clearly expressed in the mixed application.

Keywords: blue tetrazolium chloride, fertilization, manganese, *cucurbita* *Cucurbita pepo* L.

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Comment [D4]: This fragment needs revision. It is not understandable.

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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 [1]; [2]; [3] [4]; [5] [6]. In previous our publications there are data about the influence of foliar fertilizers upon the quality of fruit of marrows [7], as well as about the content of Mo in marrows [8]. Marrows are a traditional in Bulgaria early crop grown throughout the country. Interest is due to their excellent flavor and dietary qualities. In this connection it is important to trace the influence of foliar application on some components of the biological value of the fruit. In previous studies [9], [10] and [11] found correlation between foliar application and qualitative indicators of the yield.

The main aim of this study was to investigate effect of type and method of fertilization on marrows yield and fruit quality. Furthermore, we aimed at clarifying the possibility for using Blue tetrazolium chloride [12] 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.) cultivar Izobilna F, on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The soil of the field is classified as Mollic Fluvisols [13]. 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 commercially available complete foliar fertilizers with distinctly different nutrient composition were used: Fitona (7.20%N, 5.20%K₂O; 1.5%Ca 0.9%Mg, 0.1%Fe, 0.1%B, 0.01%Cu, 0.007%Zn, 0.01%Mn, 0.001%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), Humustim (on base of potassium humates-3%N, 1.14%P₂O₅, 7.83%K₂O, 3.92%Ca, 1.1%Mg, 0.001%Cu, 0.01%Zn, 0.001%Mo, 0.004%Mn 0.001%Co, B. S. Agrospeis Ltd., Bulgaria).

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Soil fertilization was carried out with NPK using a ratio $N_{160}P_{160}K_{160}$. Quantity of fertilizers was 160 kg ha^{-1} like active substance. Phosphorus ($\text{Ca}(\text{H}_2\text{PO}_4)_2$ - 46% P_2O_5); and potassium (K_2SO_4 - 50% K_2O). Fertilizers were applied with last tillage of soil before planting. Nitrogen fertilizer, introduced as NH_4NO_3 (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. For the purpose of the experiment were used by the manufacturer recommended concentrations of use. Foliar fertilizers were applied in the below given concentrations three times in the following phases - beginning of flowering, beginning of fruit production and; 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 was 600 l ha^{-1} , and in the second and third was 800 l ha^{-1} .

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 $N_{160}P_{160}K_{160}$; 6. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ Fitona; 7. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ Hortigrow; 8. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ Humustim.

Plants were cultivated according to the conventional technology for early field production of marrows, using previously produced seedlings [14]. The seedling was planted after thirty days cultivation in non-heated polythene tunnel. Plants were planted on bed-furrow surface, according to scheme $100 \times 60 / 50 \text{ cm}$ 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 [15]. Plant dry matter was determined according to the method of Stanchev et al. [15]. The content of nitrates in fruits was determined with test method GOST 29270-95 [16]. Manganese was determined in the fruits of marrows according to method described by Kostova and Mehandjiev [12]. 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 $N_{160}P_{160}K_{160}$; 6. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ Fitona; 7. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ Hortigrow; 8. Soil fertilization with $N_{160}P_{160}K_{160} + 0.3 \%$ 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 [12]. Manganese (VII) forms an ion-association complex BTC. The

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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. One gram of ~~1g from~~ the plant material was reduced to ashes in an oven in $450 - 500^\circ\text{C}$. 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. ~~It was added~~ 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 the absorbance measured at 255 nm against a reagent blank using a Spectrophotometer UV-VIS (Germany) [12]. The obtained results ~~were~~are compared with those of the AAS (Atomic absorption spectrometry) method using a Flame Atomic Absorption Spectrophotometer "Perkin Elmer" (Germany).

Statistical analysis: The results from the determination of total carbohydrates and dry matter were elaborated using the dispersion analysis method for one factor field trial [17], using the program BIOSTAT (ANOVA). Relative Standard Deviation about Mn was determined according to the BTC method.

3. RESULTS AND DISCUSSION

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 (Table 1). An exception can be discerned in fertilization with (Fitona + soil fertilization) and soil fertilization $\text{N}_{160}\text{P}_{160}\text{K}_{160}$.

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

№	Variants	Mn, mg kg^{-1} BTC method	*RSD BTC method	**Dry matter , %	AAS-Mn mg kg^{-1} AAS method	***Yield kg ha^{-1}
1	Control - non fertilized	9.67	1.9	6.02	9.80	26210c
2	0.3 % Fitona	8.32	1.5	6.97	8.00	30840c
3	0.3 % Hortigrow	9.70	1.2	6.18	9.55	32430c
4	0.3 % Humustim	8.69	1.7	6.45	8.80	39170c
5	$\text{N}_{160}\text{P}_{160}\text{K}_{160}$	8.48	2.0	4.80	8.65	38270c
6	$\text{N}_{160}\text{P}_{160}\text{K}_{160} + 0.3 \%$ Fitona	8.63	1.4	4.41	8.55	39270c
7	$\text{N}_{160}\text{P}_{160}\text{K}_{160} + 0.3 \%$ Hortigrow	10.10	1.9	5.94	10.30	40840c
8	$\text{N}_{160}\text{P}_{160}\text{K}_{160} + 0.3 \%$ Humustim	8.85	1.7	5.70	8.67	49450c

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

Comment [D10]: Numbers should not be used at the beginning of sentence.

Comment [D11]: It should be written in passive voice.

Comment [D12]: Now there are no carbohydrates in this table; they are on Fig. 1!

Comment [D13]: What was the base in calculation of Mn content? Was it FW (fresh weight) or DM (dry matter)? It should be written after kg^{-1} . The same concerns Mn content measured by AAS method.

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117 ** Dry matter, % - LSD 0.064, P=5%, LSD 0.085, P=1%; LSD 0.110, P=0.1%;
118 ***Yield kg ha⁻¹ - LSD 121.378, P=5%, LSD 163.059, P=1%; LSD 214.425, P=0.1%;
119

120 The change of the dry matter content in the fruits may be due to the increased
121 activity in the production of biomass and their priority allocation to the product portion and in
122 direct connection with the increase in the weight of the fruit. These results are in line with
123 studies of the effect of foliar fertilization on the dynamics of accumulation of dry matter by
124 [18] in fruits of marrows.

125 Manganese is an important nutritious element for plants that has an influence on the
126 quality of yield and production. In this respect the influence of manganese upon the yield of
127 marrows in the two kinds of fertilization (foliar fertilization and mixed fertilization) is
128 represented on Table 1. The experimental data show that the highest content of Mn can be
129 seen in foliar fertilization with Hortigrow and mixed fertilization N₁₆₀P₁₆₀K₁₆₀ + Hortigrow. The
130 researches showed that in the control (non fertilized), the content of manganese is lower as
131 in foliar fertilization and also in mixed fertilization.

132 The content of manganese in the fruit of marrows is highest after fertilization with
133 Hortigrow - foliar fertilization and mixed fertilization (Table 1.) Hortigrow comprises amino
134 acids that stimulate growth, development and assimilation of nutritious substances including
135 microelements. Content of amino acids is the main difference between Hortigrow and
136 Fitona responsible for the differences detected. The distinction in the content of manganese
137 in the two treatments of fertilization is 0.40 mg kg⁻¹. Hence, the additional introduction of N, P
138 and K in soil has small influence on the accumulation of manganese in the fruit of marrows.

139 The interaction between soil and foliar fertilization increased plant productivity. Early and
140 total yield was the highest after simultaneous soil fertilization with N₁₆₀P₁₆₀K₁₆₀ and foliar application
141 with 0.3% Humustim. Therefore appropriate from agronomic and economic point of view is
142 combination of soil fertilization and foliar application of 0.3% Hortigrow and 0.3% Fitona (Table 1.).
143 The highest yield is obtained in fertilization with Humustim (mixed fertilization and foliar
144 fertilization). This may be due to the fact that in the composition of the organic foliar fertilizer
145 Humustim the basic nutritious elements are humic acids that exhibit hormone activity which
146 stimulates growth and development. In independent foliar fertilization the highest yield 39170
147 kg ha⁻¹ is obtained in the content of Mn 8.69 mg kg⁻¹ in the fruit of marrows. In mixed
148 fertilization the highest yield 49450 kg ha⁻¹ is obtained in the content of Mn 8.85 mg kg⁻¹ in
149 the fruit of marrows. The experimental data show that in mixed fertilization, yield is with
150 10000 kg ha⁻¹ higher than that in foliar fertilization. It makes an impression that the content of
151 manganese is almost equal for the two ways of fertilization in which the highest yield is

Comment [D15]: Should it be 'connection of soil and foliar fertilization'?

Comment [D16]: What does mean 'early yield'?

Comment [D17]: I do not understand this. Why Hortigrow or Fitona? The best result gives Humustim. Why it is more appropriate to obtain lower yielding?

152 obtained. Consequently the main factor for higher yield is the different fertilization and not
153 the content of manganese.

154 In a recent review, [6] it was stated that the foliar uptake is due to the ability of
155 leaves and stems to absorb the nutritious elements taken in the form of certain solutions.
156 The effect of the foliar nutrition takes place much promptly in comparison to the soil
157 fertilization, which reduces the risks of physiological diseases of plants caused by deficiency
158 or scarcity of a given element [5].

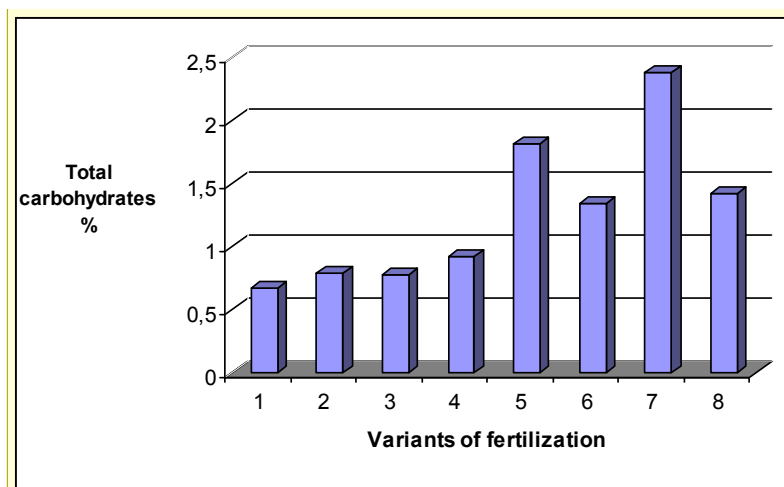
159 In the same treatments of fertilization the content of total carbohydrates in the fruit of
160 marrows has been determined. The content of total carbohydrates is the highest in plants
161 receiving soil and foliar fertilization ($N_{160}P_{160}K_{160}$ + Hortigrow). The experimental data (Fig. 1.)
162 show that the content of total carbohydrates is the highest (2.39 %) after mixed fertilization.
163 After only foliar fertilization, the content of total carbohydrates is 0.78%, or three times less
164 than in mixed fertilization. The content of total carbohydrates in the control (non fertilized) is
165 even lower (0.67%). The content of carbohydrates is the highest when we used the foliar
166 fertilizer Hortigrow together with the soil fertilization ($N_{160}P_{160}K_{160}$), a result that paralleled
167 that observed for the manganese content in fruits.

168 The experimental data show that the content of total carbohydrates does not change
169 in leaf fertilization, whereas in mixed fertilization their content is higher, as the highest value
170 of total carbohydrates can be read in fertilization with $N_{160}P_{160}K_{160}$ + Hortigrow (Fig. 1.). This
171 may be due to the higher percentage content of nitrogen, phosphorus and potassium in the
172 leaf fertilizer Hortigrow. Results are in line with established specificities in the content of total
173 sugars in the fruits of marrows from [18]. They were established that nitrogen and potassium
174 fertilization influence on the content of total sugars. These conclusions are supported by
175 research of [18] and [19]. This all indicates that the nutritious elements N, P and K that have
176 been provided additionally have positive influence on the accumulation of carbohydrates in
177 the fruit of marrows.

Comment [D18]: I do not understand this sentence.

Comment [D19]: This paragraph doesn't suit in this place. It is not related to neighboring paragraphs.

Comment [D20]: This fragment should be re-written and merged with the previous sentence. Now is repetition.



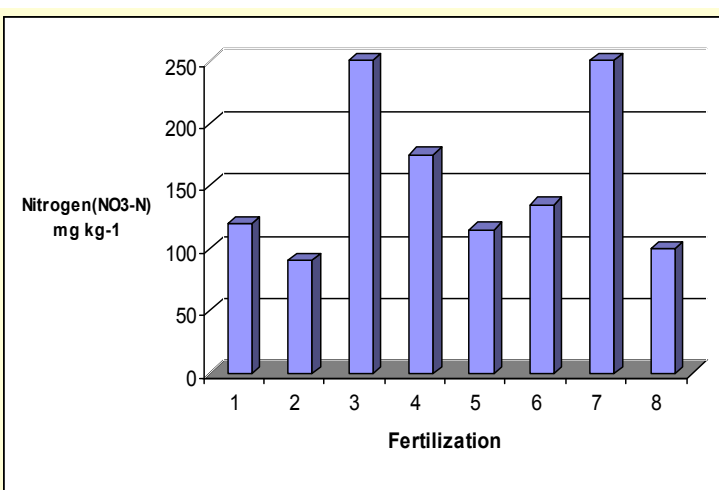
Comment [D21]: Total carbohydrates were calculated on dry matter or on fresh weight? It should be written on graph or below it.

Fig. 1. Subordination between the content of Total carbohydrates in the fruit of marrows and fertilization:-

1- Control, 2- Fitona, 3- Hortigrow, 4- Humustim, 5- Fertilization with $N_{160}P_{160}K_{160}$, 6- $N_{160}P_{160}K_{160}$ + Fitona, 7- $N_{160}P_{160}K_{160}$ + Hortigrow, 8- $N_{160}P_{160}K_{160}$ + Humustim

Total carbohydrates % - LSD 0.079, P=5%, LSD 0.107, P=1%; LSD 0.140, P=0.1%;

The influence of fertilization upon nitrate content in the fruit of marrows is presented on Fig 2. The highest nitrate content (250 mg kg^{-1}) in the fruit can be seen in the leaf fertilizer Hortigrow, as well as in mixed fertilization $N_{160}P_{160}K_{160}$ + Hortigrow. This can be explained with higher percentage content of nitrogen in the leaf fertilizer Hortigrow.



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Comment [D22]: Similarly as in Table 1 and Fig. 1, it should be written what was the base in calculations of nitrate content. Is nitrate content expressed on FW or on DM?

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Fig. 2. Subordination between the content of nitrate in the fruit of marrows and fertilization.

1- Control, 2- Fitona, 3- Hortigrow, 4- Humustim; 5- $N_{160}P_{160}K_{160}$, 6- $N_{160}P_{160}K_{160}$ + Fitona, 7- $N_{160}P_{160}K_{160}$ + Hortigrow, 8- $N_{160}P_{160}K_{160}$ + Humustim

4. CONCLUSION

The results show that application of foliar fertilizers Fitona, Hortigrow and Humustim and increased yields of marrows in preserved and improved product quality, the dry matter, total sugars and content of nitrates in fruits. The effect occurred more strongly when it was applied on a background of soil fertilization.

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 marrows 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 trend is more clearly expressed in the mixed application.

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ACKNOWLEDGEMENTS

The authors would like to thank the Research Fund of the Agricultural University for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

'Author DK' and 'Author DH' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author DK' and 'Author DM' managed the analyses of the study. 'Author DH' managed the literature searches. All authors read and approved the final manuscript.

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Comment [D23]: This fragment needs revision because it is not understandable.

Comment [D24]: One of the aims of the research was 'to clarify the opportunity for using Blue tetrazolium chloride (BTC) as a reagent for determination of micro quantities of manganese in plant samples.' What about this?

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272 | **APPENDIX**

Comment [D25]: Reference list is still prepared
NOT ACCORDING to the Journal requirements!

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