

1\*, 1, 2,  
, , 1 ,  
1 , " 4,  
37000 ,  
2 , 32000 ,

## Organic Production of the Pepper by Using of Cow Manure and Retort Beech Charcoal

Sanja Živkovi<sup>1\*</sup>, Tanja Vasi<sup>1</sup>, Darko Jevremovi<sup>2</sup>,  
Sonja Filipovi<sup>1</sup>

<sup>1</sup>Agriculture faculty, Kosaničeva 4, 37 000 Kruševac, University of Niš, Serbia

<sup>2</sup>Fruit Research Institute, Kralja Petra I 9, 32 000 Rača, Serbia

\*E-mail: gajicsanja43@gmail.com

Original scientific paper

### SUMMARY

The objective of this study was to

- research the charcoal application in vegetable farming under organic conditions.
- The experiment was established in the greenhouse designed by random block system with pepper genotype Amanda, in treatment with cow manure and retort beech charcoal and control with cow manure without retort beech charcoal.
- The experiment was set up at Trnare locality, Kruševac municipality in a greenhouse according to a random block system in 2 replicates, planting 25 plants treated with cow manure and retort beech charcoal and 25 plants treated only with cow manure as a control. Pepper were grown on a single tree.

The aim was to determine the influence of the applied material on the number of fruits per plant and the weight of the fruit directly affecting the yield in the organic

27.35%

- growing system. The parameter that is followed is pepper yield under temperate climatic conditions. The following parameters were monitored in the experiment: fruit yield per plant and number of fruits per plant. In the treatment with retort beech charcoal, the Amanda genotype had an average yield 27.35% higher than the control plants.

**Key words:** fruit, organic cultivation, retort beach charcoal, pepper, yield

(*Capsicum annuum* L.)

## INTRODUCTION

Pepper (*Capsicum annuum* L.) is of great economic importance in our country. It is one of the most significant cultures. It has high nutritional and biological value.

It can be used in many different ways, such as fresh, sour, baked, frozen, supplemented with various dishes and in the industry it is a raw material for different types of processing. It belongs to the group of intensive vegetable crops. It requires a great deal of human work involvement. It is a highly accumulative crop because it generates high income per unit area. Initially grown as an ornamental and then a herb, pepper has over time become one of the most important vegetable crops in the world.

Today, peppers are most grown in Asia, Europe, and America. The large pepper fruit is mostly grown in Spain, Serbia, Montenegro, China, USA, Bulgaria, Hungary, Italy, Russia, Ukraina, France and Greece. It is mainly grown in a temperate zone, somewhat tropical, and in Europe it is the culture of the south and the protected area of the north. In the national economy of the country, these vegetables are of great importance, both for the nutrition of the population and for international trade, because apart from fresh exports, various products of peppers as well as spices are exported (Tepi , 2009).

(Tepi , 2009).

(Gvozdenovi et al., 2006).  
 2017 ., 17  
 386,00 ha 11.4 t ha<sup>-1</sup>  
 (webrzs.stat.gov.rs, 2018).  
 (Gvozdenovi et al., 2006).  
 (PH = 6-7)

The importance of peppers also arises on the surfaces on which they are grown. It's a very intense culture. It is grown in an open field and in a protected area. Due to its multiple uses in the diet as well as in the processing industry, it is considered a highly regarded vegetable.

Depending on the variety and the purpose of production, it is used in technological (usable) or physiological (botanical) maturity in fresh and processed state (Gvozdenovi et al., 2006).

In 2017, 17,386.00 ha were sown under these vegetables in Serbia, with an average yield of 11.4 t ha<sup>-1</sup> (webrzs.stat.gov.rs, 2018). Depending on the type of growth, pepper can be produced in different ways and used for different purposes.

Since peppers have long vegetation, it is necessary to provide nutrients in an accessible form throughout the growing season. Nutrition should ensure a balanced supply of plants with nutrients during growth and development, especially in times of increased water demand. Continuous irrigation increased nutrient leaching and increased plant nutrient uptake (Gvozdenovi et al., 2006).

Peppers require fertilizers, nitrogen, phosphorus and potassium fertilizers. Peppers respond neutral (PH = 6-7) to slightly acidic. It is extremely sensitive to saline lands. Due to its long vegetation, irrigation and relatively poorly developed root system, the pepper, which is in a shallow plowed layer, has high requirements for organic and mineral nutrients.

Therefore, all nutrients must be cured in an easily accessible form in the root zone.

Nutrient requirements are throughout vegetation and especially during flowering, fruit formation and ripening stages (Gvozdenovi et al., 2006).

(Gvozdenovi et al., 2006).

(Lehman et al., 2005; Biederman and Harpole, 2013).

(Chan et al., 2007).

(Mann, 2005).

(PCM).

( / )

( )

( ) CO<sub>2</sub>,

( , )

2018).

(Hagemann et al.,

- Organic farming combines tradition, innovation and science in order to produce healthy product and keep the environment protected. The use of activated carbons in organic production is one of the possibilities to preserve and raise the soil quality and therefore the yield of the cultivated plants (Lehman et al., 2005; Biederman and Harpole, 2013).

- Soil biochar amendment is based on two thousand years old experience, which in recent decades has been renewed because of proven multiple benefits (Chan et al., 2007). This importance is largely long-term, but also reveals the short-term effects (Mann, 2005).

- Both biochar and activated carbon are pyrogenic carbonaceous materials (PCM). They are produced by thermochemical conversion of carbonaceous feedstock (pyrolysis or/and activation). Biochar is produced from sustainably sourced biomass and is used for non-oxidative applications in agriculture (e.g., in the soil) and is also discussed as a raw material for industrial processes. By definition, it is used for carbon sequestration.

- Hence, if “biochar” is used as a fuel, it is burned and the carbon is transformed (oxidized) into CO<sub>2</sub>, it is actually classified as charcoal. Activated carbon is produced from any carbon source (fossil, waste or renewable) and engineered to be used as sorbent to remove contaminants from both gases and liquids.

- Both materials have their distinct history, widely separated scientific communities and separated bodies of literature. Unfortunately, a generally accepted terminology and definition is lacking (Hagemann et al., 2018).

- However, as the proposed

(Laird 2008; Woolf et al., 2010; Hagemann et al., 2018 ; Yadav et al., 2018).	-	applications of biochar and activated carbon increasingly overlap, awareness of the "other" domain in each case can be beneficial. Nowadays both biochar and activated carbon are used for soil remediation, which before has been solely an application of activated carbon. When the activated carbon is not removed after the application and if this activated carbon was produced from renewable feedstock and is complying to further specifications, it can be considered as biochar (Laird 2008; Woolf et al., 2010; Hagemann et al., 2018; Yadav et al., 2018).
(Šeremeši et al., 2015; Tian et al., 2018; Yadav et al., 2018). (2018)	-	Many studies confirmed that soil incorporated with biochars can improve plant growing (Šeremeši et al., 2015; Tian et al., 2018; Yadav et al., 2018). According to Tian et al., (2018) biochar incorporation induces soil alkalization which can increase soil nitrification and nitrogen (N) levels. Increases in soil pH are likely to affect electrical conductivity (EC), cation exchange capacity (CEC) and increase alkaline metal ( $Mg^{2+}$ , $Ca^{2+}$ and $K^+$ ) oxides.
pH  (N).  (EC),  ( $Mg^{2+}$ , $Ca^{2+}$ $K^+$ ).	-	Likewise, it reduces soluble forms of aluminum, which is suggested as the most significant biochar factor affecting P solubility (De Luca et al., 2009; Tian et al., 2018; Yadav et al., 2018).
(De Luca et al., 2009; Tian et al., 2018; Yadav et al., 2018).	-	Beneficial effects of biochar have been elaborated in studies world wide. However, there is a lack of experimental confirmation of the biochar application in our agricultural science. Researches of biochar use have been mainly conducted on soils under tropical and humid climatic conditions, which are more degraded and have a lack of soil organic carbon (Šeremeši et al., 2015; Tian et al., 2018; Yadav et al., 2018).
a.	-	Therefore, the aim of this study is to research the charcoal application in vegetable farming under organic conditions. The parameter that is followed is pepper yield under temperate climatic
(Šeremeši et al., 2015; Tian et al., 2018; Yadav et al., 2018).	-	

(Vasi , 2016).  
 (

7 998 ha,  
 1549 ha. 2014 .  
 2%

25

40 cm,  
 30 cm.  
 Amanda  
 20 2019 .  
 20 2019 .

200 kg 60 m<sup>2</sup>

g/ 200 g/ 200

200 g/

conditions. For the organic production, it is very important to choose the right genotype to be grown according to ecological principles (Vasi , 2016). The total area under certification (taking into account the organic status of the plots and plots in the conversion period) in Serbia is 7,998 ha, plus meadows and pastures of 1,549 ha. In 2014, vegetables accounted for only 2% of the certified plant species.

**MATERIAL AND METHODS**

The experiment was set up at Trm are locality, Kruševac municipality in a greenhouse according to a random block system in 2 replicates, planting 25 plants treated with cow manure and retort beech charcoal and 25 plants treated only with cow manure as a control.

The peppers were planted in the rafters. The plants were pinched on two branches, as a binder used as a support for the pepper plants.

The row spacing was 40 cm, while the plant spacing in one row was 30 cm. The production of pepper Amanda genotype seedlings has been started on 20 March, 2019 in the glasshouse, and the seedling has been done on 20 May, 2019 in the greenhouse. Before sowing, the soil was prepared by adding cow manure in the amount of 200 kg per 60 m<sup>2</sup> at the depth of the sowing layer. Seven days before planting, a mixture of cow manure in the amount of 200 g/plant and 200 g/plant of retort beech charcoal was introduced at the depth of the sowing layer. The control was not treated with retorted beech charcoal, but only with cow manure in the amount of 200 g/plant. The activated charcoal used for this purpose was produced from natural raw materials and obtained by carbonation of beech, selected according to strictly defined technical requirements by activation of steam in a static furnace.

Due to its organic origin and

233-750 mg/g),

(  
N, P,  
K, (mg/kg: Ca-8590, Mg-  
1260, K-7400, P-380, S-350 Mn-32, Fe-  
230, Zn-20, Cu-23),

0-2.5 mm.

, pH

9-11.

(ANOVA)  
Statistica 8

0.05.

2125.00 g

1543.00 g

( 1).

production method, charcoal has a certain degree of activity (iodine number of 233-750 mg/g), which allows it to retain water reserves and thus provide the moisture needed by the plant.

The use of activated carbon in organic production is very useful because of the introduction of N, P, K, trace elements (mg/kg: Ca-8590, Mg-1260, K-7400, P-380, S-350 Mn-32, Fe-230, Zn-20, Cu-23), organic substances, humic acids and amino acids that help the plant's level and improve their health. The granulometric range of the material ensures that the soil is loose because it does not dissolve in the soil. Activated carbon granulometry 0-2.5 mm was used in this experiment. The ashes of the material thus obtained have a high level of elements such as oxides of potassium, calcium and magnesium.

Also, activated charcoal contains a higher percentage of charcoal so it has phosphates in ash, which is an excellent source of this microelement for the plant. Due to the origin and content of alkali metals, the pH of this material is 9-11.

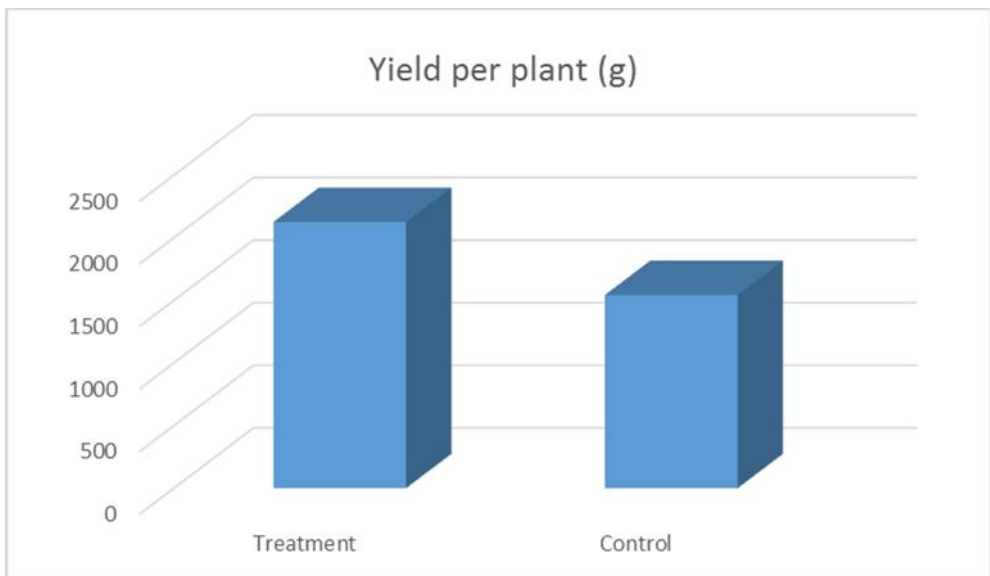
Peppers were grown on a single tree. The following parameters were monitored in the experiment: fruit yield per plant and number of fruits per plant.

Analysis of variance (ANOVA) in Statistica 8 statistical program was used to examine differences in the measured characteristics between treated and untreated pepper plants and their interaction, using the Student's T-test of significance level 0.05. The results were presented in a graph.

## RESULTS

The yield of treated plants averaged 2,125.00 g per plant and in the control plants averaged 1,543.00 g (Figure 1). Fruit mass is a genotype characteristic and is one of the factors

that determine its purpose.



**Fig. 1. Effect of treatment and control on yield of the fruit (grams per plant)**

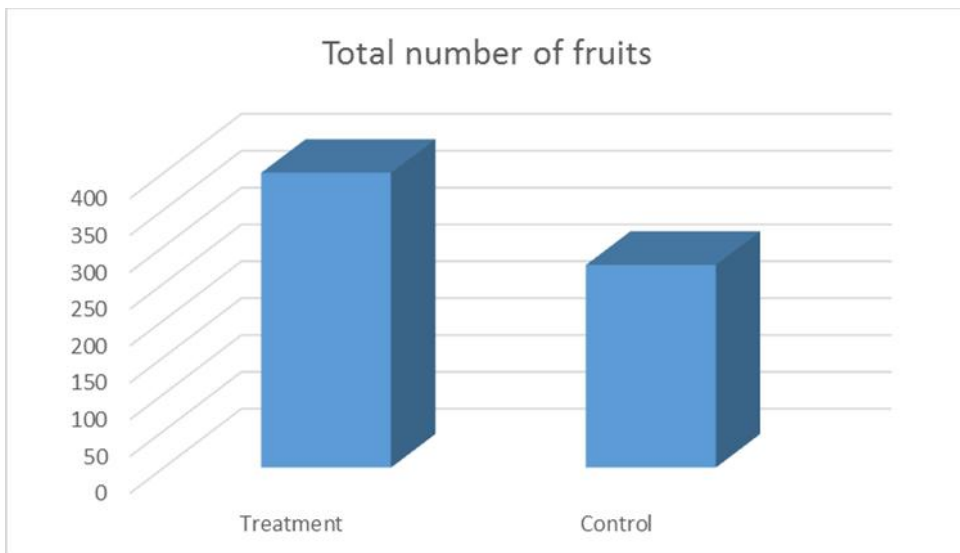
(ANOVA) ( ) , , , ( ) , , ( 2 3).

Analysis of variance (ANOVA) for fruit yield (grams per plant) indicated a statistically significant difference between plants treated with retorted beech charcoal and non-treated plants as well as their interactions. Specifically, it was found that the plants from the treatment had a statistically significantly higher fruit yield (in grams per plant) than the plants from the control.

It was also found that there was a statistically significant difference between the total fruit yield and the number of fruits on the pepper plants under treatment compared to the control plants (Figures 2 and 3).







. 3.

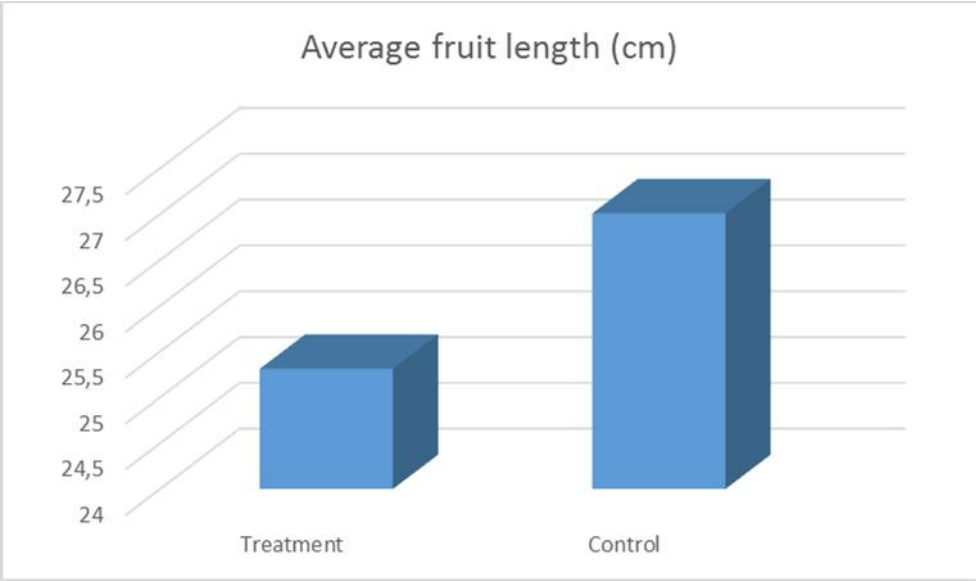
**Fig. 3. Effect of treatment and control on total number of fruits**

, - . , -  
( 1, 2 3).  
56 348.00 g,  
- 40 940.00 g.  
( 4, 5).

- The differences in yield between treatment and control within each individual harvest were statistically significant, with the smallest difference within the last harvest. On average, in treatment and control, the highest yield was recorded at the last harvest (Figures 1, 2 and 3).

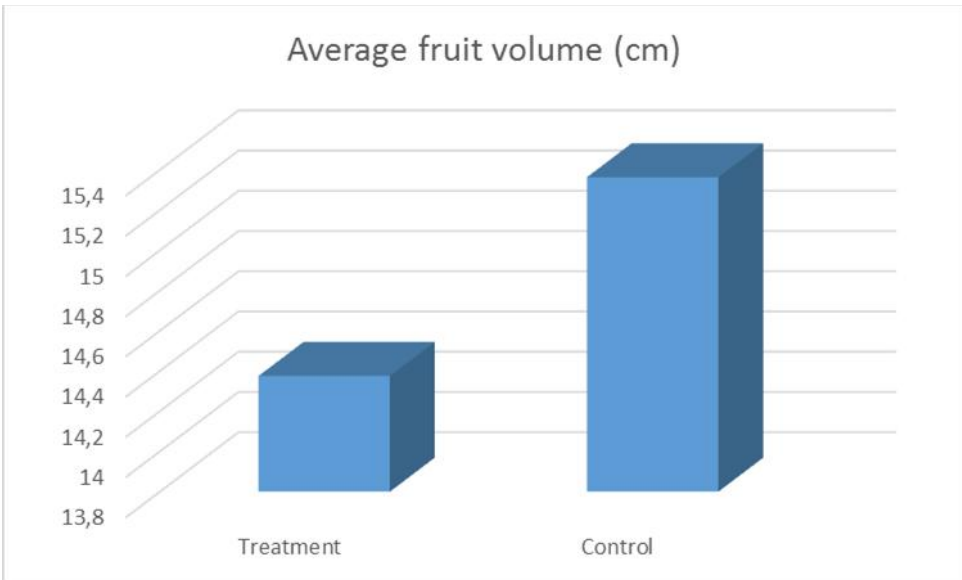
The total fruit yield for the pepper plants under treatment was 56,348.00 g and for the control plants 40,940.00 g.

We also monitored the dimension of the fruits and after statistical data processing it was observed that the fruits from the treatment were smaller than the fruits from the control (Figure 4, 5).



. 4.

**Fig. 4. Effect of treatment and control on the dimension of pepper fruit**



. 5.

**Fig. 5. Effect of treatment and control on the dimension of tomato fruit**

## DISCUSSION

(Rylski et al., 1994).  
 Bogdanovi et al. (2012),  
 -  
 ,  
 :  
 ,  
 ,  
 -  
 (NPK). -  
 . Fertor  
 ,  
 .  
 -  
 ,  
 (NPK),  
 -  
 .  
 Deni (2010)  
 Bogdanovi et al. (1995)  
 NO3-N  
 ( Bogdanovi  
 et al., 2012).  
 ,  
 ,  
 -  
 .  
 28  
 7  
 2019.

Optimal temperatures and brightness in the early stages of development determine the yield and quality of the fruit (Rylski et al., 1994).

According to Bogdanovi et al. (2012) of applied organic fertilizers, the highest yields of pepper of the Amfora variety, on and without foil, were achieved with: fertilizer system, then composted pig manure, earthworm, burnt bovine manure, and the lowest only with mineral fertilizers (NPK). The greater effect of fertilizer on pepper yield compared to other applied organic fertilizers results from the technology of production of this fertilizer and its composition. Fertor is pelleted organic chicken manure that has been added to other organic materials of plant origin and contains all biogenic elements.

Also, treatments using composted pig manure resulted in higher pepper and foil yields than gluten, burnt cow manure and mineral fertilizers (NPK), which could be explained by the much higher solubility and presence of biogenic elements, primarily nitrogen, from composted pig manure, relative to other fertilization systems.

According to research by Deni (2010) on maize and Bogdanovi et al. (1995) on wheat the effect of NO<sub>3</sub>-N from liquid pig manure in the year of application to yield was identical to the effect of applied mineral fertilizers (loc. cit. Bogdanovi et al., 2012).

In this study, it was found that plants treated with beech retort charcoal had a higher total fruit yield per plant as well as compared to control plants.

Thus, for the plants from the treatment the first harvest was already on July 28, while for the plants from the control the first harvest was on August 7, 2019. Fruit yield is conditioned primarily by genetic polygenic factors, but is also dependent

	<ul style="list-style-type: none"> <li>- on the external environment. It is clear from this study that beech retort charcoal has a positive effect on tomato yield in the greenhouse.</li> </ul>
	<p>The largest are the fruits of the first floor, and after that the size decreases. Bouquets of pepper varieties are matured and can also be harvested multiple times. From fertilization to technological maturity, depending on the variety and growing conditions, it usually takes about 30 days.</p>
<p>30</p> <p>( )</p>	<ul style="list-style-type: none"> <li>- Peppers can also be harvested at physiological (botanical) ripeness, when it has a red, orange or purple fruit color.</li> <li>- Such fruit maturity is most commonly used in industrial processing. Pepper fruit yield at physiological maturity is often 50% lower than that achieved at technological maturity.</li> </ul>
<p>50%</p> <p>30%.</p> <p>(Gvozdenovi et al., 2006).</p>	<ul style="list-style-type: none"> <li>- With the delay in harvesting the fruit, the yield can also be reduced by 30%. Medium late and late production, which is characteristic of our area, arrives for harvest in late July and early August, and takes place until the occurrence of frosts (Gvozdenovi et al., 2006).</li> </ul>
<p>Yadav et al. (2018)</p> <p>59</p> <p>57</p>	<p>There are no data in the literature on the effect of retorted beech charcoal on pepper yield.</p> <p>Positive crop and biomass yield was found for biochar produced from wood, paper pulp, wood chips and poultry litter. Yadav et al. (2018) reviewed published data from 59 pot experiments and 57 field experiments from 21 countries and found crop productivity increased by 11% on average.</p>
<p>11%. Yadav et al. (2018)</p> <p>30 t ha<sup>-1</sup></p> <p>(30%), (14 %)</p> <p>(29%)</p>	<p>Also, Yadav et al. (2018) found benefits at field application rates typically below 30 t ha<sup>-1</sup> field application and reported that increases in crop productivity varied with crop type with greater increases for legume crops (30%), vegetables (29%), and grasses (14%) compared to cereal crops corn (8%), wheat (11%), and rice</p>

(11%)	(7%).	(8%),	(7%). These data are consistent with the results of this paper.
Yamato et al. (2006)	-	-	According to Yamato et al. (2006) maize production was significantly increased after the application of bark charcoal under a fertilized condition in an infertile soil environment. A positive effect of biochar addition on maize dry biomass could be ascribed to higher soil N-retention that was observed by Baronti et al. (2010).
Baronti et al. (2010).	-	-	These are only preliminary results. Further detailed investigations should be undertaken in order to find the most optimal amount and time of application of beech retort charcoal in crops under the climatic conditions of Serbia.

## CONCLUSIONS

The highest fruit yield was achieved on activated carbon plants, while the yield on control plants was 27.35% lower. This study indicates the positive impact of retorted beech charcoal on the yield of pepper plants in the greenhouse.

## / REFERENCES

1. **Baronti, S., G. Alberti, G.D. Vedove, F.D. Gennaro, G. Fellet, L. Genesio, F. Miglietta, A. Peressotti and F.P. Vaccari**, 2010. The Biochar Option to Improve Plant Yields: First Results from Some Field and Pot Experiments in Italy, *Italian J. Agron.*, 5: 3-12.
2. **Biederman, L.A. and W.S. Harpole**, 2013. Biochar and Its Effects on Plant Productivity and Nutrient Cycling: a Meta-analysis, *GCB Bioenergy*, 5: 202-214.
3. **Bogosavljevi , D., Ž. Ilin and R. abilovski**, 2012. Uticaj Sistema ubrenja i Nastiranja Zemljišta na Prinos Paprike Sorte Amfora. *Letopis nau nih radova*, 36(1), 53-62.
4. **Chan, K.Y., L. Van Zwieten, I. Meszaros, A. Downie, S. Joseph**, 2007. Agronomic Values of Green- Waste Biochar as a Soil Amendment, *Australian J. Soil Res.*, 45: 629-634.
5. **De Luca, T.H., M.D. Mac Kenzie and M.J. Gundale**, 2009. Biochar Effects on Soil Nutrient Transformation. In: J Lehmann, S Joseph (Ed.), *Biochar for Environmental Management Science and Technology*. Earthscan, London, pp. 251-280.
6. **Gvozdenovi , ., D. Bugarski, A. Taka and J. ervenski**, 2006. Proizvodja Povrtarske Paprika na Otvorenom Polju iz Rasada. Nau ni institut za ratarstvo i povrtarstvo. *Zbornik radova, sveska*, 42: 241-258.

7. **Hagemann, N., K. Kurt Spokas, H.P. Schmidt, R. Kägi, M.A. Böhler, D. Thomas and T.D. Bucheli**, 2018. Activated Carbon, Biochar and Charcoal: Linkages and Synergies across Pyrogenic Carbon's ABCs. *Water*, 10, 182; doi:10.3390/w10020182 [www.mdpi.com/journal/water](http://www.mdpi.com/journal/water).
8. <http://webrzs.stat.gov.rs> (accessed March 2020).
9. **Laird, D.**, 2008. The charcoal Vision: A win-win-win Scenario for Simultaneously Producing Bioenergy, Permanently Sequestering Carbon, while Improving Soil and Water Quality. *Agron. J.*, 100: 178-181.
10. **Lehmann, J., J. Gaunt and M. Rondon**, 2005. Bio-char Sequestration in Terrestrial Ecosystems – a review, *Mitig. Adapt. Strat. Glob. Change*, 11: 403-427.
11. **Mann, C.C.**, 2005. New Revelations of the Americas before Columbus. Vintage and Anchor Books, New York pp. 1-576.
12. **Rylski, I., B. Aloni, L. Karni and Z. Zaidman**, 1994. Flowering, Fruit Set, Fruit Development and Fruit Quality under Different Environmental Conditions in Tomato and Pepper Crops. *Acta Hortic*, 366: 45-56.
13. **Tepi , A.**, 2009. Karakteristike Oleorizina Mlevena Za inske Paprika Dobijenog Klasi nom i Ekstrakcijom Superkriti nim Ugljen-dioksidom. Doktorska disertacija, Tehnološki fakultet, Novi Sad, pp. 1-145.
14. **Tian, X., C. Li, M. Zhang, Y. Wan, Z. Xie and B. Chen**, 2018. Biochar Derived from Corn Straw Affected Availability and Distribution of Soil Nutrients and Cotton Yield. PLoS ONE 13(1): e0189924. <https://doi.org/10.1371/journal.pone.0189924>.
15. **Wolf, D., J.E. Amonette, F.A. Street-Perrott, J. Lehmann and S. Joseph**, 2010. Sustainable Biochar to Mitigate Global Climate Change. *Nature comm.* 1: 56.
16. **Yadav, N.K., K. Vijay, K.R. Sharma, R.S. Choudhary, T.S. Butter, G. Singh, M. Kumar and R. Kumar**, 2018. Biochar and Their Impacts on Soil Properties and Crop Productivity: a review. *Journal of Pharmacognosy and Phytochemistry*, 7(4), 49-54.
17. **Yamato, M., Y. Okimori, I.F. Wibowo, S. Anshori and M. Ogawa**, 2006. Effects of the Application of Charred Bark in Acacia Mangium on the Yield of Maize, Cowpea, Peanut and Soil Chemical Properties in South Sumatra, Indonesia. *Soil Sci. Plant Nutr.*, 52: 489-495.
18. **Šeremeši , S., M. Živanov, D. Milošev, J. Vasin, V. iri and M. Vasiljevi , N. Uji** , 2015. Effects of Biochar Application on Morphological Traits in Maize and Soybean. *Zbornik Matice srpske za prirodne nauke. Matica Srpska J. Nat. Sci.*, Novi Sad, N 129, 17-25.

1\* , 1 , 2 ,  
1 , 1 ,  
1  
2 " , 1331 , 1407 ,

## Yield and Fruit Quality of Greenhouse Tomato in Response to Different Level of Irrigation and Fertilization

Galina Patamanska<sup>1\*</sup>, Ivanka Mitova<sup>1</sup>, Daniela Miteva<sup>2</sup>,  
Antoaneta Gigova<sup>1</sup>

<sup>1</sup>Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Pushkarov",  
1331 Sofia, Bulgaria

<sup>2</sup>Institute of Cryobiology and Food Technology, 1407 Sofia, Bulgaria

\* -mail: patamanska\_g@yahoo.com

Original scientific paper

### SUMMARY

Solanum lycopersicum 'Big Beef',  
2019 .  
: V<sub>1 0</sub> –  
; V<sub>1 1</sub> –  
N<sub>8.95</sub> P<sub>11.82</sub> K<sub>13.87</sub>; V<sub>1 2</sub> –  
N<sub>11.59</sub> ;  
P<sub>15.84</sub> K<sub>17.74</sub>; V<sub>1 3</sub> –  
N<sub>14.50</sub> P<sub>20.13</sub> K<sub>1.88</sub>; V<sub>2 0</sub> –  
(60% ) ; V<sub>2 1</sub> –  
N<sub>8.95</sub> ; V<sub>2 2</sub> – deficit irrigation;

In this study, the effect of optimal and deficit irrigation by applying increasing rates of fertilization on yield and fruit quality of tomato (Solanum lycopersicum 'Big Beef') cultivated under drip fertigation with mulch in unheated tunnel greenhouse was tested. The experiment was conducted in the Chelopechene experimental field of the Institute of soil science, agrotechnologies and plant protection in town of Sofia, Bulgaria in 2019. The following treatments were tested: V<sub>1 0</sub> – full irrigation at irrigation rate estimated by evapotranspiration; V<sub>1 1</sub> – full irrigation, fertilization N<sub>8.95</sub> P<sub>11.82</sub> K<sub>13.87</sub>; V<sub>1 2</sub> – full irrigation, fertilization N<sub>11.59</sub> P<sub>15.84</sub> K<sub>17.74</sub>; V<sub>1 3</sub> – full irrigation; fertilization N<sub>14.50</sub> P<sub>20.13</sub> K<sub>1.88</sub>; V<sub>2 0</sub> – deficit irrigation (60% ); V<sub>2 1</sub> – deficit irrigation, fertilization N<sub>8.95</sub> P<sub>11.82</sub> K<sub>13.87</sub>; V<sub>2 2</sub> – deficit irrigation;



$P_{11.82}$   $K_{13.87}$ ;  $V_{2\ 2}$  -  
 $N_{11.59}$   $P_{15.84}$   $K_{17.74}$ ;  
 $V_{2\ 3}$  -  
 $N_{14.50}$   $P_{20.13}$   $K_{1.88}$ .

$V_{1\ 3}$  - 80.4 t/ha,

40%

$V_{2\ 0} - V_{2\ 3}$   
 $V_{1\ 0} - V_{1\ 3}$

2018 .

916 h ,

2,08 %

74 %  
(MAFF, 2019).

(Patane et

fertilization  $N_{11.59}$   $P_{15.84}$   $K_{17.74}$ ;  $V_{2\ 3}$  - deficit irrigation, fertilization  $N_{14.50}$   $P_{20.13}$   $K_{1.88}$ .

The yield and quality of tomatoes were determined by treatments. The experimental results show that the amount of water supplied for irrigation and fertilizer input affect the tomato productivity. The highest yield was obtained from the treatment  $V_{1\ 3}$  - 80.4 t ha<sup>-1</sup>, at which the highest irrigation and fertilizer rate were applied. Reducing the irrigation rate by 40% leads to lower yields for the  $V_{2\ 0}$  -  $V_{2\ 3}$  deficit irrigation treatments compared to the  $V_{1\ 0}$  -  $V_{1\ 3}$  full irrigation treatments. In the physicochemical analyses performed, higher values of total soluble solids, reducing sugars, vitamin C and titratable acidity were obtained for tomatoes grown under deficit irrigation compared to those under full irrigation.

**Key words:** tomato, drip irrigation, fertigation, yield, fruit quality, greenhouse

## INTRODUCTION

In recent years, tomato has gained widespread popularity worldwide as a healthy food that can help reduce the risk of certain human diseases, including cancer. For Bulgaria they are a traditional vegetable and a favourite food.

Tomatoes are one of the most important crops cultivated in greenhouses. Although the total greenhouse area in Bulgaria was within 916 ha in 2018, which was only 2.08% of total vegetable area, because of the high intensity of greenhouse production, the share of greenhouse tomatoes is almost 74% of those produced in the country. (MAFF, 2019).

Tomato has high water requirements (Patane et al., 2011) and irrigation is needed during the cultivation.

al., 2011).

- In greenhouse cultivation, the irrigation system is only source for water supply and the question of its effectiveness is of particular interest.

- In recent years, drip irrigation systems have been widely used in the greenhouses, which allow supply of the needed water for irrigation to the plants directly into the active soil layer without surface and deep water losses, with minimal losses of evaporation and filtration.

- Drip irrigation with the application of mineral fertilizers (fertigation) during the vegetation period of the plants leads to high yields.

- Inefficient use of fertilizers can result in compromised yields, deteriorating soil fertility, quality of the production and cause serious environmental damage.

- More studies worldwide are dedicated to the effects of drip fertigation on the yield of tomato and the fruit quality. (Du et al., 2017, Wang and Xing, 2017).

(Du et al., 2017, Wang and Xing, 2017).

- The aim of this study was to investigate the effect of optimal and deficit irrigation by applying increasing rates of fertilization on yield and fruit quality of tomato (*Solanum lycopersicum* 'Big Beef') cultivated under drip fertigation with mulch in unheated tunnel greenhouse.

## MATERIAL AND METHODS

- The study was conducted in the "Chelopechene experimental field of the Institute of soil science, agrotechnologies and plant protection in town of Sofia, Bulgaria in an unheated polyethylene tunnel-type greenhouse with dimensions of 7.9 x 53 m and a total area of 420 m<sup>2</sup> in 2019. The experimental field with geographical coordinates: 42 ° 44 22.8 N, 23 ° 28 3.7 E is a part of the Sofia Field, located at 550 m above sea level. This area has

7.9 53 m

420 m<sup>2</sup>.

: 42°44 22.8 N, 23°28 3.7 E,

550 m.

(*Solanum lycopersicum* "Big Beef").

:  $V_1$  -

( )  $V_2$  -

60%

4 : 0 -

$N_{8.95}$

$N_{11.59}$

$N_{14.50}$

$P_{11.82}$   $K_{13.87}$  ,  $N_{11.59}$   $P_{15.84}$   $K_{17.74}$  ,  $N_{14.50}$   $P_{20.13}$   $K_{21.88}$  ,  $N_{8.95}$   $P_{11.82}$   $K_{13.87}$  ,  $N_{11.59}$   $P_{15.84}$   $K_{17.74}$  ,  $N_{14.50}$   $P_{20.13}$   $K_{21.88}$  .

:  $V_1T_0$ ,  $V_1T_1$ ,  $V_1T_2$ ,  $V_1T_3$ ,  $V_2T_0$ ,  $V_2T_1$ ,  $V_2T_2$ ,  $V_2T_3$ .

24 m<sup>2</sup>

81

0.6

0.5

m

m.

60

1.5 l/h.

cm

+UV 15 mic/1.20 m.

1-2 l

- 7

3-7

continental climate characterized by cold winter. The soil type of the experimental site is Chromic Luvisol, which can be defined as moderate to strong water-permeable with an average filtration capacity.

The object of the study is tomato variety (*Solanum lycopersicum* "Big Beef").

A two-factor experiment was performed with experimental factors - irrigation and fertilization. The factor irrigation was applied in two levels:  $V_1$  - full irrigation at irrigation rate estimated by evapotranspiration ( ),  $V_2$  - deficit irrigation (60% ). The factor fertilization was applied at four levels.  $0$  - without fertilizer,  $1$  - suboptimal fertilization  $N_{8.95}$   $P_{11.82}$   $K_{13.87}$  ,  $2$  - optimal fertilization  $N_{11.59}$   $P_{15.84}$   $K_{17.74}$  ,  $3$  - luxury fertilization  $N_{14.50}$   $P_{20.13}$   $K_{21.88}$  .

The following treatments were tested:  $V_1T_0$ ,  $V_1T_1$ ,  $V_1T_2$ ,  $V_1T_3$ ,  $V_2T_0$ ,  $V_2T_1$ ,  $V_2T_3$ ,  $V_2T_4$ .

The experimental treatments were arranged according to the method with long plots. Each plot has a surface of 24 m<sup>2</sup> and consisted of twin rows of tomato with a total of 81 plants. They are planted „checkerboard“ at a spacing of 0.6 m and at a distance between rows of 0.5 m.

Irrigation was performed with a drip irrigation system, comprising a command unit and two batteries consisting eight laterals situated next to the each row of tomato. The laterals were simple 1.3 l/h dripper lines with a 60 cm emitters spacing. The volume of water supplied to the batteries was controlled by water meters mounted on the main pipelines. Mulching was applied to further reducing the evaporation. Black polyethylene mulch (UV 15 mic/1.20 m) was used.

Immediately after planting the tomatoes in a permanent place, a watering of 1-2 l per plant was carried out to intercept seedlings and next watering 7 days later. Depending on the growth stage of tomatoes, watering was carried out with a frequency of 3-7 days. Deficit irrigation began to be applied from the

beginning of fruit formation.

$V_1T_0, V_1T_1, V_1T_2, V_1T_3,$

Irrigation rate for the full irrigation treatments  $V_2T_0, V_2T_1, V_2T_3, V_2T_4$  was determined by the sum of daily evapotranspiration values for the irrigation interval. For each day of the vegetation period the tomato evapotranspiration was determined by the formula:

$$ET = kc ET_o \tag{1}$$

$ET_o$  is reference evapotranspiration, (mm) and  $k$  is crop coefficient.

The reference evapotranspiration was calculated using the Penman-Monteith method (Allen et al., 1998):

(Allen et al., 1998):

$$ET_o = \frac{0.409\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \tag{2}$$

$ET_o$  is reference evapotranspiration rate, (mm day<sup>-1</sup>),  $T$  is mean air temperature (°C),  $\Delta$  is slope of the saturated vapor pressure curve (kPa/°C);  $R_n$  is net radiation flux (MJ m<sup>-2</sup>day<sup>-1</sup>);  $G$  is sensible heat flux into the soil (MJ m<sup>-2</sup>day<sup>-1</sup>);  $\gamma$  is psychrometric constant (kPa/°C);  $u_2$  is wind speed at 2 m above the ground (m s<sup>-1</sup>);  $e_a$  is mean daily ambient vapor pressure (kPa) and  $e_s$  is mean saturated vapor pressure (kPa).

Meteorological parameters necessary for the calculation: temperature, relative humidity of the air and the solar radiation in the greenhouse, were measured using an automatic weather station and recorded in a data logger (HOBO USB Micro Data Logger, USA). The micro station is located in the center of the greenhouse. The average monthly values of the parameters of the microclimate in the greenhouse and evapotranspiration during the growing season of tomato are shown in Table 1.

Micro Data Logger, USA).

1.

The values of the crop coefficient for the various stages of the tomato

development were selected according to literary data.

1.

**Table 1. Average microclimatic conditions in greenhouse during the tomato growing season and evapotranspiration**

/ Month	Solar radiation, W/m <sup>2</sup>	Temperature		RH mean, %	ET <sub>0</sub> , mm month <sup>-1</sup>
		Min, °C	Max, °C		
/ June	160.05	13.75	36.62	67.57	4.07
/ July	141.67	13.63	36.14	63.28	4.04
/ August	134.36	13.73	36.99	54.04	4.06

kg/ha K<sub>2</sub>O. 450 kg/ha P<sub>2</sub>O<sub>5</sub> 500 (450 kg/ha). 100% (N, P, K) (Mn, Mg, B, Cu, Ca). (Fe, Zn, K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, 27% K<sub>2</sub>O 18% N, 11% P<sub>2</sub>O<sub>5</sub>, 59% K<sub>2</sub>O, kg/ha. MixRite 2.5 (kg) t.ha<sup>-1</sup>.

In the autumn, storage fertilization with 450 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 500 kg ha<sup>-1</sup> K<sub>2</sub>O was carried out. When planting tomatoes, nitrogen fertilization with ammonium nitrate (450 kg per ha) was performed. During the growing season of tomato simultaneously with irrigation, 100% water-soluble fertilizers were introduced, which contain macronutrients (N, P, K) and microelements (Fe, Zn, Mn, Mg, B, Cu, Ca).

Depending on the growth stage of tomato were applied: after planting tomato - mineral fertilizer containing 16% N, 69% P<sub>2</sub>O<sub>5</sub> and 16% K<sub>2</sub>O, during their vegetative development - fertilizer containing 27% N, 27% P<sub>2</sub>O<sub>5</sub>, 27 % K<sub>2</sub>O and in the period of fruiting the applied fertilizer contains 18% N, 11% P<sub>2</sub>O<sub>5</sub>, 59% K<sub>2</sub>O, at change of the fertilizer rate from 20 to 60 kg per ha. In order to apply the exact fertilizer rate, a MixRite 2.5 hydraulic fertilizer injector and a water meter were used.

Yield and fruit quality - during the period of fruiting of the tomatoes harvests per treatment were carried out, picking up all the fruits that have reached full maturity.

The yield of tomatoes is read by weight (kg) and recalculated in t.ha<sup>-1</sup>. In

:  
 „Kern“;  
 pH- „Hanna“;  
 0,1nNaOH;  
 2,6-  
 ;  
 :  
 (ANOVA).  
 SPSS Statistics.

three representative harvests, average samples of tomato fruits are taken per treatment, and for determination of the quality physicochemical analyzes are performed for determination of the following indicators: total soluble solids - with moisture analyser Kern; active acidity - with Hanna pH meter; titratable acidity - by titrimetric method with 0,1nNaOH; vitamin C - by the Moore method - titration with 2,6-dichlorophenol-indophenol; reducing sugars - by the Shoorl method.

Statistical methods: The studied parameters were analyzed by two-way analysis of variance (ANOVA). The analysis of variance was calculated using the software package SPSS Statistics.

## RESULTS AND DISCUSSION

:  
 4.41 mm/day.  
 3-4 9  
 2.

Daily evapotranspiration of tomato was determined based on data on the microclimate parameters in the greenhouse: temperature, humidity and solar radiation, and the irrigation rate - on the basis of the sum of daily evapotranspiration for the period between irrigations. The average value of evapotranspiration of tomatoes is 4.41 mm day<sup>-1</sup>. Half of the eight treatments were full irrigation treatments, and the others four – deficit irrigation treatments. Depending on the stage of development of the tomato, thirty-six irrigations were carried out with a frequency of 3-4 days and 9 fertigations with increasing fertilizer rates. Experimental results for tomato yield per treatment showing the influence of irrigation and fertilization with different irrigation and fertilizer rates are given in Table 2.

2.

**Table 2. Total yield per treatment**

	/ Irrigation	/ Fertilization	/ Total Yield, t/ha
100% Tc		T <sub>0</sub>	69.03
		T <sub>1</sub>	71.01
		T <sub>2</sub>	72.93
		T <sub>3</sub>	80.41
60% Tc		T <sub>0</sub>	47.08
		T <sub>1</sub>	47.10
		T <sub>2</sub>	50.82
		T <sub>3</sub>	65.98

- V<sub>1</sub>T<sub>0</sub> - V<sub>1</sub>T<sub>3</sub>,  
 - V<sub>1</sub>T<sub>3</sub> - 80.4 t/ha,  
 - 40% V<sub>2</sub>T<sub>0</sub> - V<sub>2</sub>T<sub>3</sub>  
 31.8 %, 33.7%, 30.3% 18%  
 V<sub>1</sub>T<sub>0</sub> - V<sub>1</sub>T<sub>3</sub>,  
 V<sub>2</sub>T<sub>3</sub>,  
 - 65.98 t/ha. 18%  
 e 31.8%  
 3  
 , pH,  
 ,  
 pH,  
 ,  
 pH

Highest yields were obtained in the full irrigation treatments V<sub>1</sub>T<sub>0</sub> - V<sub>1</sub>T<sub>3</sub>, in which the optimal irrigation regime was realized. The highest yield of tomato was obtained from treatment V<sub>1</sub>T<sub>3</sub> - 80.4 t ha<sup>-1</sup>, in which the highest fertilizer rate was applied. The reduction of the irrigation rate by 40% leads to lower yields for the treatments V<sub>2</sub>T<sub>0</sub> - V<sub>2</sub>T<sub>3</sub> by 31.8%, 33.7%, 30.3% and 18%, respectively, compared to the full irrigation V<sub>1</sub>T<sub>0</sub> - V<sub>1</sub>T<sub>3</sub> treatments. And in deficit irrigation treatments the highest yield was in the treatment V<sub>2</sub>T<sub>3</sub>, fertilized with the maximum fertilizer rate - 65.98 t ha<sup>-1</sup>. The yield obtained for this treatment is 18 % lower than full irrigation treatment with maximum fertilization, while the yield for the deficit irrigation treatment without fertilization is 31.8% lower than that for full irrigation without fertilization.

Table 3 shows the average values of the parameters total soluble solids, pH, reducing sugars, vitamin C and titratable acidity obtained by physico-chemical analyzes of tomato fruits.

It can be seen that the fruit quality parameters of greenhouse tomato were influenced by the irrigation levels. With the exception of the pH parameter, all parameters have higher values for tomato grown under deficit irrigation than those fully irrigated. For the pH parameter, higher values were found in the full

irrigation treatment.

3.

**Table 3. Fruit quality parameters as affected of irrigation and fertilization levels**

	T Fertilization (T)	/ Irrigation (V)		ANOVA		
		100%	60%	V	T	V*T
Total soluble solids, %	T <sub>0</sub>	7.20	8.00	*	ns	ns
	T <sub>1</sub>	8.17	8.69			
	T <sub>2</sub>	7.00	9.86			
	T <sub>3</sub>	7.82	8.48			
PH / Acidity	T <sub>0</sub>	4.04	4.01	ns	ns	ns
	T <sub>1</sub>	4.07	4.00			
	T <sub>2</sub>	4.11	4.04			
	T <sub>3</sub>	4.15	4.09			
Reducing sugars, %	T <sub>0</sub>	1.64	1.74	ns	ns	ns
	T <sub>1</sub>	1.49	1.78			
	T <sub>2</sub>	1.46	1.41			
	T <sub>3</sub>	1.36	1.82			
Vitamin C, mg %	T <sub>0</sub>	32.34	32.82	**	ns	ns
	T <sub>1</sub>	25.83	32.56			
	T <sub>2</sub>	26.05	33.15			
	T <sub>3</sub>	29.27	37.75			
Titratable acidity, %	T <sub>0</sub>	0.25	0.43	*	ns	ns
	T <sub>1</sub>	0.35	0.50			
	T <sub>2</sub>	0.33	0.46			
	T <sub>3</sub>	0.26	0.35			

\*,\*\* / significant at p< 0.05, or p< 0.01; ns

/ non significant.

Analysis of variance of experimental data revealed that the level of the irrigation has a pronounced effect on three of the indicated quality parameters: dry matter, vitamin C, titratable acidity (Table 3).

Deficit irrigation has a significant effect (p 0.05) on the total soluble solids of tomato fruits. The increase in the total soluble solids content in the tomatoes grown under deficit irrigation is 8-10% compared to the full irrigation treatments. Other authors (Birhanu et al., 2010) have reported similar results.



(Birhanu et al., 2010).

20%

< 0.01.

(Yang et al., 2017; Grancharova et al., 2018).

5 %.

(Ripoll et al., 2016).

Higher concentration of vitamin C in tomato fruits by up to 20% was observed in the treatments with deficit irrigation compared to the full irrigation treatments, and this difference was statistically proven at a significance level of  $p < 0.01$ . Similar results have been obtained in previous studies, the reason is the lower accumulation of water in the fruits (Yang et al., 2017; Grancharova et al., 2018).

With regard to the titratable acidity parameter, higher values were also obtained in the deficit irrigation treatments, as the increase compared to the full irrigation treatments was on average about 30%, with a statistically proven difference of 5%.

A higher average content of reducing sugars was observed in the deficit irrigation treatments compared to the full irrigation treatments, but the statistical studies did not show statistically significant differences. Other studies have also shown that more sugars accumulate in tomatoes under deficit irrigation (Ripoll et al., 2016).

The statistical tests showed no significant difference between the average in terms of factors fertilization and the interaction of both factors irrigation and fertilization.

**CONCLUSIONS**

Solanum lycopersicum „Big Beef“

An experiment was conducted in a greenhouse for growing tomato variety Solanum lycopersicum "Big Beef" under drip fertigation and an assessment of the impact of optimal and deficit irrigation with the application of increasing fertilizer rates on yield and quality of tomato was made.

Experimental results show that the level of irrigation and the amount of fertilizer applied affect the productivity of tomatoes. The most suitable for the good plant development and obtaining a high

(100% ETc),	-	-	yield is full irrigation (100% ETc), as the highest yield of tomatoes was obtained in the treatment in which the highest fertilizer rate was applied - 80.4 t ha <sup>-1</sup> .
- 80.4 t/ha. (60% ETc)	-	-	Deficit irrigation (60% ETc) leads to lower yields compared to full irrigation.
t/ha,	-	-	The highest yield was in the treatment with application of the maximum fertilizer rate - 65.98 t ha <sup>-1</sup> , which is 18% lower than in full irrigation, i.e. with a 40% reduction in irrigation and a luxurious fertilization, the yield decreased by 18% compared to full irrigation.
18%	-	- 65.98	
40 %	-	-	
18 %	-	-	Statistical analysis of the experimental results obtained for quality parameters showed that irrigation has a greater impact than fertilization on the quality of tomato production, and moderate deficit irrigation can greatly improve it.
	-	-	Physico-chemical analyses showed higher values of total soluble solids, reducing sugars, vitamin C and titratable acidity for tomatoes grown under deficit irrigation than those grown under full irrigation.

## ACKNOWLEDGEMENTS

The present work is based on researches that are funded from the Bulgarian National Science Fund of the Ministry of Education and Science under a bilateral cooperation project between Bulgaria and China, on topic: "Comparison of Soil Quality in Protected Cultivation for Sustainable Agriculture in China and Bulgaria".

## / REFERENCES

1. Allen, R.G., L.S. Pereira, D. Raes and M. Smith, 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. *Irrigation and Drainage Paper 56*, FAO, Rome.
2. Birhanu, K. and Ketema Tilahun, 2010. Fruit Yield and Quality of Drip-irrigated Tomato under Deficit Irrigation. *African Journal of Food, Agriculture, Nutrition*

*and Development*, 10 (2), 2139-2151.

3. **Du, Y., H. Cao, S. Liu, X. Gu and Y. Cao**, 2017. Response of Yield, Quality, Water and Nitrogen Use Efficiency of Tomato to Different Levels of Water and Nitrogen under Drip Irrigation in Northwestern China. *Journal of Integrative Agriculture*. 16 (5), 1153-1161.
4. **Grancharova, El., G. Patamanska, G. Kostadinov, A.Gigova, D. Miteva. I. Nacheva and K. Dimov**, 2018. Quality of Greenhouse Tomatoes Cultivated under Different Irrigation Levels. *Journal of Mountain Agriculture on the Balkans*, 21(4), 257-264.
5. MAFF, 2019. Annual report on the situation and development of agriculture (Bg).
6. **Patane, C., S. Tringali and O. Sortino**, 2011. Effects of Deficit Irrigation on Biomass, Yield, Water Productivity and Fruit Quality of Processing Tomato under Semi-arid Mediterranean Climate Conditions. *Sci. Hortic.*, 129, 590-596.
7. **Ripoll, J., L. Urban, . Brunel and . Bertin**, 2016. Water Deficit Effects on Tomato Quality Depend on Fruit Developmental Stage and Genotype. *Journal of Plant Physiology*, 190: 26-35.
8. **Wang, X. and Y. Xing**, 2017. Evaluation of the Effects of Irrigation and Fertilization on Tomato Fruit Yield and Quality: a Principal Component Analysis. *Scientific reports*, 7(1), 350.
9. **Yang, H., H. Cao, X. Hao, L.Guo, H. Li and X. Wu**, 2017. Evaluation of Tomato Fruit Quality Response to Water and Nitrogen Management. *International Journal of Agricultural and Biological Engineering*, 10(5), 85-94.

\*,

, 4108

## Effect of Application of Microbial Fertilizers on Nutrient Status of Virginia Tobacco

Radka Bozhinova\*, Tsveta Hristeva

Tobacco and Tobacco Products Institute, 4108 Markovo, Bulgaria

\*E-mail: rbojinova@yahoo.com

Original scientific paper

### SUMMARY

The effects of different microbial fertilizers (containing arbuscular mycorrhizal fungi *Glomus*, *Trichoderma* and plant growth-promoting bacteria *Bacillus*) on soil properties and concentration of macro- and micronutrients in Virginia tobacco have been studied in field experiment set on Cleyic-Chromic Luvisol in 2018. Three microbial products were used: Europlus® (Italy), Micotric L® (Italy) and Rhizo-Vam Basic® (Germany). The experimental design was a randomized complete block replicated three times. It was found that soil properties and plant nutrient levels were influenced by the use of microbial products. A comparison of unfertilized treatment indicated that with the exception of manganese and zinc, concentrations of macro- and microelements in Virginia tobacco leaves were significantly increased by the application of Europlus. The Mn and Zn concentration was significantly higher by Rhizo-Vam Basic addition. There was a positive effect of

Micotric L

Micotric L application on concentrations of nitrogen, potassium, calcium, magnesium and manganese in leaves, but differences with non-fertilized treatment were not significant. The results show that microbial fertilizers used in this experiment can have a positive effect on the soil, the plants, and therefore the environment. Additional research is necessary in order to have better understanding of plant-soil-microorganisms interactions and these microbial products to be ecologically acceptable alternative to chemical fertilizers.

**Key words:** microbial fertilizers, tobacco, soil, macronutrients, micronutrients

## INTRODUCTION

Fertilization plays key roles in tobacco yield and quality. Chemical fertilizers containing mainly nitrogen, phosphorus and potassium are used in the Virginia tobacco cultivation. Excessive use of mineral fertilizers can have agronomic, environmental, and economic consequences.

Because of the over use of chemical fertilizers, the soil environment used for tobacco planting gradually deteriorates, and the quality of tobacco leaves also decreases (Shi et al., 2009). In order to reduce the negative effects on the environment, and to increase soil fertility, agrarian policies at European and world level envisage limiting the use of chemical substances for both fertilization and plant protection (Tsvetkov et al., 2017b).

Plant growth promoting micro-organisms (PGPM) are an important group of microbial inoculants, which exist in rhizosphere and have the ability to inhabit the root of the plants and improve their development. Their positive influence is achieved through solubilization of phosphorus, nitrogen fixation, production

(Shi et al., 2009).

(Tsvetkov et al., 2017b).

(PGPM),

al., 2018). (Stamenkovi et al., 2012) (FZB42) Cheng et al.

Tsvetkov et al. (2017a) *Glomus* *Trichoderma*

*Trichoderma* – N Fe, Mg. *Glomus*

2005). (Wu et al., 2005). (N, P K) N

*Glomus*, *Trichoderma* . *Bacillus*)

of plant nutrients and phytohormones, protection from pathogens and recovery from stressful environmental conditions.

This is the main reason for the increasing usage of many PGPMs which formulations are commonly known as microbial fertilizers (Stamenkovi et al., 2018). The microbial fertilizer (FZB42) could obviously promote the growth of tobacco plants, increase the expanding extent and economic characters of tobacco leaves, and improve the internal quality and smoking quality of tobacco leaves (Cheng et al., 2012). Tsvetkov et al. (2017a) reported that the variants, including mycorrhizal fungi *Glomus* and *Trichoderma* demonstrated best mineral uptake rates: the variant *Glomus* – in N and Fe accumulations, *Trichoderma* – in Mg ones. The application of biofertilizer containing mycorrhizal fungus and three species of bacteria significantly increased the maize growth and microbial inoculum not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil (Wu et al., 2005).

Studies on the impact of the microbial fertilizers in the cultivation of tobacco are limited. Hence, the aim of this study was to investigate the effect of different microbial products on the agrochemical properties of soil and concentration of macro- and micronutrients in Virginia tobacco.

## MATERIAL AND METHODS

The effects of microbial fertilizers on soil properties and concentration of macro- and micronutrients in Virginia tobacco (Virginia 0514) have been studied in field experiment set on Cleyic-Chromic Luvisol in 2018.

The experimental design was a

0514), 2018 . ( -  
 (Cleyic-Chromic Luvisol). -  
 ,  
 .  
 110 cm - 40 cm  
 (2250 /da). ( -  
 , )  
 .  
 :  
 Europlus®, EUROVIX,  
 (*Glomus* spp.+ *Bacillus* spp.);  
 Micotric L®, EUROVIX,  
 (*Glomus* spp.+ *Trichoderma* spp.);  
 Rhizo-Vam Basic®,  
 (*Glomus intraradices*).  
 (Europlus – 0,4 g/ , Micotric L – 4,4  
 ml/ Rhizo-Vam Basic – 13,3  
 g/ )  
 .  
 .  
 ( -  
 ).  
 : ( 2 ) - ,  
 -  
 ;  
 -  
 2N HCl. -  
 Ca Mg 1N  
 KCl.  
 Fe, Mn, Zn Cu  
 0,005 M DTPA + 0,1 M , = 7,3.  
 N, P, K, Ca,  
 Mg, Fe, Mn, Zn Cu  
 .  
 ,  
 .  
 12 h 75 ° .  
 .

randomized complete block replicated three times. Plants were spaced at 110 cm in rows and 40 cm apart (22500 plants ha<sup>-1</sup>).

Cultural practices such as irrigation, weeding, pest and disease control were applied in accordance with the recommended practices for commercial plantations.

The following commercial products were used as the source of mycorrhizal fungi (MF) and plant growth-promoting bacteria (PGPR):

Europlus®, EUROVIX, Italy  
 (*Glomus* spp.+ *Bacillus* spp.);  
 Micotric L®, EUROVIX, Italy  
 (*Glomus* spp.+ *Trichoderma* spp.);  
 Rhizo-Vam Basic®, Germany  
 (*Glomus intraradices*).

One week after transplanting, the microbial products (Europlus – 0.4 g per plant, Micotric L – 4.4 ml per plant and Rhizo-Vam Basic – 13.3 g per plant) were incorporated in the top soil layer.

In August (when tobacco was at full flowering stage), soils from the different treatments were sampled for studying effects of microbial fertilizers on soil fertility. Soil samples were collected from the upper layer (0-25 cm) of each plot. The following soil characteristics were determined: pH in water, N<sub>min</sub> (NH<sub>4</sub>+NO<sub>3</sub>), available P – by the Olsen method, available K – in 2N HCl. Available Ca and Mg were determined by using 1N KCl. A solution of 0.005 M DTPA+0.1 M TEA, pH 7.3 was used for extraction of the Fe, Mn, Zn and Cu mobile forms from soil.

The concentration of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in mature leaves from middle stalk position was determined. All samples were washed with tap water to remove any adhering soil particles and rinsed afterwards with distilled water. Following drying at 75°C for 12 h samples were ground. Total nitrogen in the plants was determined by the Kjeldahl method. The preparation of

plant samples for analysis of P, K, Ca, Mg, Fe, Mn, Zn and Cu was made by means of dry ashing and dissolution in 3 M HCl. Phosphorus was determined colorimetrically by the molybdovanadate procedure. An atomic absorption spectrometer „SpektrAA 220”, Australia was used for determination of K, Ca, Mg, Fe, Mn, Zn and Cu content in the soil and plant samples.

Results were analyzed using the SPSS statistical package for Windows and differences were assessed with the Duncan’s multiple range test at the 0.05 probability level.

## RESULTS AND DISCUSSION

The effect of the application of microbial fertilizers on selected soil properties is presented in Table 1.

1.

**Table 1. Soil properties as dependent on application of microbial fertilizers**

Treatment	(2)	N <sub>min</sub> (mg/kg)	2 5 (mg/100 g)	2 (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)
Control*/	* 6.76a**	11.92a	1.02c	48.82b	87.4c	47.4ab
Europlus	6.68a	8.94a	1.72bc	51.12b	110.6b	44.6b
Mic tric L	6.64a	11.92a	2.22b	54.06a	109.3b	45.6b
Rhizo-Vam Basic	6.63a	9.24a	3.35a	50.78b	132.3a	50.0a

\* Control - without application of microbial fertilizer

\*\* ( $<0.05$ );

\*\* Different letters within each column indicate that the means are significantly different ( $P<0.05$ )

The data showed that by comparison with the control, soil pH<sub>(H2O)</sub> values were unaffected by the addition of microbial fertilizers. Among various nitrogen forms, ammonia nitrogen and nitrate nitrogen play the most important role when seen from the ecological perspective. The amount of mineral nitrogen available to plants depends, among others, on the type and rate of fertilizers (Schmidt et al., 2000; S dej and Przekwas, 2008). The mineral nitrogen content in soil under different treatments varied from 8.9 to 11.9 mg kg<sup>-1</sup>. No significant differences were determined



mg/kg,

Micotric L Rhizo-Vam Basic.

Micotric L.  
Ca

DTPA-

2.

between control plot and treatments with application of microbial fertilizers. Soil-available P showed an increase with application of microbial fertilizers. The differences in available P between the control treatment and the Mic tric L and Rhizo-Vam Basic applications were significant. The addition of Mic tric L led to significant increases in the soil-available K<sub>2</sub>O content compared to the unfertilized soil. The soil Ca concentration was enhanced by the application of microbial fertilizers. There were no significant differences in Mg content between control plot and treatments with fertilization.

The concentrations of DTPA-extractable metals in the soil are presented in Table 2.

2.

Fe, Mn, Zn Cu

**Table 2. Content of available Fe, Mn, Zn and Cu in the soil**

Treatment /	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Control* / *	30.0b**	14.67b	12.61a	22.50b
Europlus	28.75b	17.15b	11.85a	28.55a
Mic tric L	30.6b	17.92b	11.83a	21.15b
Rhizo-Vam Basic	34.9a	19.37a	13.26a	27.27a

\* Control - without application of microbial fertilizer

\*\* Different letters within each column indicate that the means are significantly different (P < 0.05);

\*\* Different letters within each column indicate that the means are significantly different (P < 0.05)

MAFF (Mitsios et al. 2005)

Mn,  
Fe

Fe Mn

Rhizo-Vam Basic.

Cu

Europlus Rhizo-Vam Basic.

3.

According to MAFF classification (Mitsios et al. 2005) soil was characterized as having sufficient level of available Mn, high content of available Fe and very high content of mobile zinc and copper. The soil available Fe and Mn level increased significantly with Rhizo-Vam Basic application. Microbial fertilizers resulted in a small differentiation in the available Zn content in the soil. Significantly higher Cu content was observed in plots receiving Europlus and Rhizo-Vam Basic as compared to control treatment.

The effect of the application of microbial fertilizers on macronutrient concentrations of Virginia tobacco leaves is presented in Table 3.

3.

(%)

**Table 3. Macronutrient concentrations of tobacco leaves (% of dry weight)**

Treatment /	N	P	K	Ca	Mg
Control*/	1.41b**	0.17b	1.56b	0.27b	0.56b
Europlus	2.28a	0.20a	2.62a	0.66a	0.73a
Mic tric L	1.71ab	0.15b	1.89b	0.34b	0.59b
Rhizo-Vam Basic	1.81ab	0.19ab	1.96b	0.35b	0.56b

\* Control - without application of microbial fertilizer

\*\* Different letters within each column indicate that the means are significantly different (P<0.05)

\*\* Different letters within each column indicate that the means are significantly different (P<0.05)

(1,41-2,28%)  
 Campbell (2000)  
 (1,3-2,25%).  
 N  
 Europlus.  
 ( 0,15% 0,20%)  
 Campbell (2000),  
 (0,12%-0,3%).  
 Europlus.  
 Europlus (P<0.05).  
 (2000)  
 1,5-2,5%  
 Campbell

- Differentiation of nitrogen content  
 - by variant is expressed relatively well.  
 The observed values (1.41-2.28%) are similar to those reported by Campbell (2000) for Virginia tobacco (1.3-2.25%).  
 - The N content in the leaves was the lowest in the control variant and increased in all treatment receiving microbial fertilizers. Significantly higher N concentrations in the leaves were observed at the Europlus treatment as compared to control.  
 - The concentrations of P in leaves were from 0.15% to 0.20% and are close to data observed by Campbell (2000) for the optimum phosphorus content in mature leaves (0.12%-0.3%). The application of microbial fertilizers had no pronounced effect on P content in Virginia tobacco. Only Europlus treatment significantly increased the concentration of P in the leaves.  
 - Among the three treatments with application of microbial fertilizer, Europlus variant led to highest K concentration in leaves and the differences between different treatments were significant (P<0.05). According to Campbell (2000) the concentration of K in the leaves of 1.5-2.5% can be assumed as sufficient to ensure the growth processes and to obtain satisfactory yields of Virginia tobacco. The values observed for all treatments were within the acceptable ranges.  
 - The concentration of Ca in mature leaves in all treatments was not high –

0,27% 0,66% -  
 Apostolova (1986). Mitreva and  
 Woltz (1967) McCants and  
 0,2%. ( 0,56% 0,73%)  
 - 0,2%.  
 Mg  
 Europlus,  
 Fe  
 45,4 114,6 mg/kg  
 ( 4). Campbell (2000)  
 Fe  
 mg/kg. 40-200  
 al. (2009).  
 Europlus.

from 0.27% to 0.66% and is lower than the values obtained by Mitreva and Apostolova (1986). Mg deficiency may be expected to occur when the value in the leaf is 0.2 percent or less of the dry weight (McCants and Woltz, 1967). Observed values in our experiment (0.56%-0.73%) were higher than the critical concentration of 0.2%. The good supply of plants with Mg was probably because tobacco was grown on a soil with large quantities of available magnesium. There was no a clear positive effect of fertilizer treatment on concentrations of Ca and Mg in the leaves. With the exception of Europlus treatment, concentrations of calcium and magnesium were not significantly affected by applied microbial products.

Fe concentration in mature leaves varied between 45.4 and 114.6 mg kg<sup>-1</sup> (Table 4). According to Campbell (2000) the optimum Fe content in mature tobacco leaves is from 40 to 200 mg kg<sup>-1</sup>. For the experimental conditions the measured concentrations in leaves are within these limits, but lower than those found by Golia et al. (2009). The iron concentration in leaves was significantly influenced by Europlus addition.

4.)

(mg/kg)

**Table 4. Micronutrient concentrations of tobacco leaves (mg kg<sup>-1</sup> dry matter)**

Treatment /	Fe	Mn	Zn	Cu
Control*/	54.8b**	43.2b	71.7b	19.7b
Europlus	114.6a	67.9ab	75.1b	23.6a
Mic tric L	45.4b	55.0b	70.0b	18.5b
Rhizo-Vam Basic	74.4b	91.5a	83.7a	20.9b

\* Control - without application of microbial fertilizer  
 \*\* Different letters within each column indicate that the means are significantly different (P<0.05);

Mn  
 43,2 91,5 mg/kg ( 4).  
 Jones et al. (1991)  
 Mn  
 20 400 mg/kg.  
 Rhizo-Vam Basic

The content of Mn in mature leaves was from 43.2 to 91.5 mg kg<sup>-1</sup> (Table 4). According to Jones et al. (1991) Mn content in tobacco leaves varies depending on the plant stage from 20 to 400 mg kg<sup>-1</sup>. Significantly higher concentration of Mn was recorded in the Rhizo-Vam Basic treatment as compared

83,7 mg/kg ( 4).  
 al. (2009),  
 52,6 28,8 mg/kg.  
 Rhizo-Vam Basic.  
 Cu  
 18,5 23,6 mg/kg ( 4).  
 (24-37 mg/kg),  
 Golia et al. (2009),  
 (3-10 mg/kg),  
 Campbell (2000).  
 Cu  
 Europlus.

to control variant.

The zinc content in leaves ranged from 70.0 to 83.7 mg kg<sup>-1</sup> (Table 4). These values are higher than the average values reported by Golia et al. (2009), which for the Virginia tobacco leaves at first and second priming was 52.6 and 28.8 mg kg<sup>-1</sup> respectively. High levels of zinc in leaves in the present experiment can probably be explained by the high content of available Zn in the soil. The zinc concentration in the tobacco leaves was the highest in Rhizo-Vam Basic treatment.

The content of Cu in leaves was from 18.5 to 23.6 mg kg<sup>-1</sup> (Table 4). These values were similar or lower than the average concentrations for the Virginia tobacco (24-37 mg kg<sup>-1</sup>) reported by Golia et al. (2009), but higher than the ranges (3-10 mg kg<sup>-1</sup>) reported by Campbell (2000). The Europlus treatment resulted in the significantly highest Cu concentration.

## CONCLUSIONS

Microbial fertilizers used in the study have different effects on soil properties. The addition of Rhizo-Vam Basic led to significant increases in the soil-available phosphorus, calcium, iron, manganese and copper content compared to the unfertilized soil. There was significant increase of available Ca and Cu in treatment receiving Europlus. Compared to the control, the application of microbiological product Mic tric L increased significantly the content of available phosphorus, potassium and calcium.

Plant nutrient levels were influenced by the use of microbial products. A comparison of unfertilized treatment indicated that with the exception of manganese and zinc, concentrations of macro and microelements were significantly increased by the application of Europlus. The Mn and Zn concentration

Rhizo-Vam Basic  
 Ca Cu  
 Europlus.  
 Micotric L  
 Europlus,



Plant Yields in the Long-term Eternal Rye Trial in Halle (Saale), Germany. *J. Plant Nutr. Soil Sci.*, 163, 639-648.

10. **Shi, Q.H., F. Jiao, W. Geng, Q.P. Li and X.D. Shi**, 2009. An Overview on Research into Factors Hindering Continuous Cropping in Flue-cured Tobacco. *Acta Tabacaria Sinica*, 6, 81-84.

11. **Stamenkovi, S., V. Beškoski, I. Karabegovi, M. Lazi and N. Nikoli**, 2018. Microbial Fertilizers: A Comprehensive Review of Current Findings and Future Perspectives. *Span. J. Agric. Res.*, 16(1), e09R01. <https://doi.org/10.5424/sjar/2018161-12117>

12. **Tsvetkov, I., E. Markov, T. Dzhambazova and D. Georgiev**, 2017a. Mineral Elements Uptake and Dry Matter Accumulation in Mycorrhizated Nursery Plants Gisela 6/Van. *Journal of Mountain Agriculture on the Balkans*, 20(1), 251-258.

13. **Tsvetkov, I., L. Georgieva, D. Tsvetkova, V. Michailova and D. Georgiev**, 2017b. Benefits of the Micorrhizal Fungi *Glomus* spp. for Grapevine Nutrient Uptake, Biocontrol and Microbial Ecology. *Journal of Mountain Agriculture on the Balkans*, 20(1), 227-250.

14. **Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung and M.H. Wong**, 2005. Effects of Biofertilizer Containing N-fixers, P and K solubilizers and AM Fungi on Maize Growth: a Greenhouse Trial. *Geoderma*, 125: 155-166.