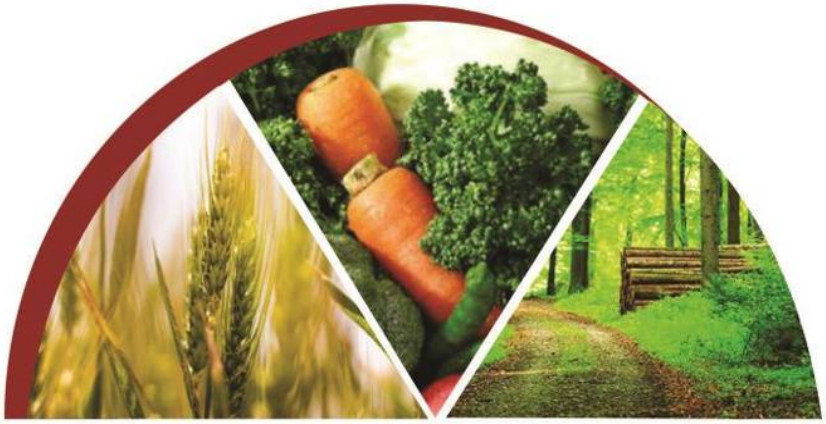
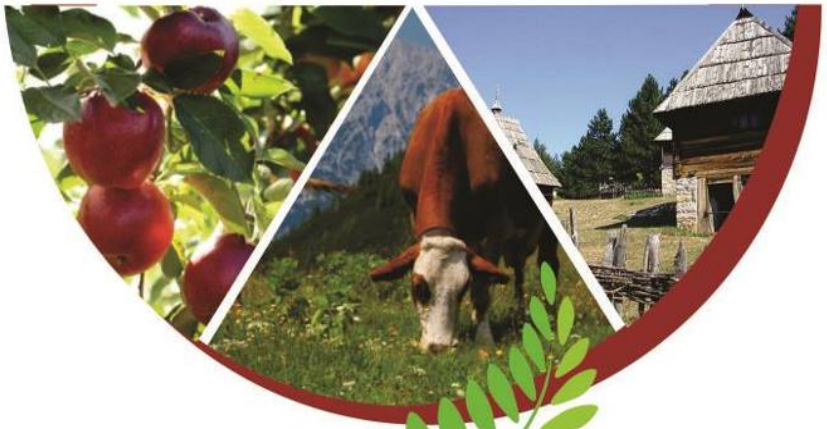
BOOK OF PROCEEDINGS

AgroSym

VIII International Scientific Agriculture Symposium

Jahorina, October 05-08, 2017





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"AGROSYM 2017"

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FOREWORD

A Word from the Editor

The successful implementation of the 2030 Agenda for Sustainable Development and the achievement of the Sustainable Development Goals depend on progress in agriculture. Agriculture has far-reaching implications in terms of poverty eradication, food security, health and wellbeing, biodiversity, climate change and economic development.

The most important goal of the 8th International Agriculture Symposium "AGROSYM 2017" – held in Jahorina on 05-08 October, 2017 – was to promote sustainability principles in agriculture. Sustainable agriculture is an important element of the overall effort to make human activities compatible with the demands of the earth's eco-system. Thus, an understanding of the different approaches to ecological agriculture is necessary if we want to utilize wisely the planet's resources. One of the goals of the sustainable agriculture movement is to create farming systems that eliminate, or at least mitigate, environmental harms associated with industrial agriculture. That aim can be realized only with context-specific agro-ecological practices; these depend on regional characteristics, climate conditions, soil types as well as socio-cultural, institutional and political settings.

AGROSYM 2017 made an important contribution to agriculture science and practice. Symposium themes cover all branches of agriculture and are divided into seven sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environment protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agro-forestry.

During the four-day Symposium approximately 250 papers were presented orally and 1030 as posters. The contributions, representing the current research in different countries, were presented to more than 1300 participants from more than 85 countries. We are encouraged by the great success of this year's edition of AGROSYM 2017.

This publication is comprised of an edited selection of over 400 papers submitted to AGROSYM 2017. Each paper included in the present Proceedings was positively reviewed by two referees. Full texts of the submitted communications are also available online (http://www.agrosym.rs.ba). Some selected papers will be published in AGROFOR International Journal (http://www.agrofor.rs.ba/), International Journal "Agriculture and Forestry" (www.agricultforest.ac.me) and EcoPersia (www.ecopersia.modares.ac.ir). Many thanks to all the authors, reviewers, session moderators and colleagues for their help in editing the Proceedings. The results reported here will contribute to the dissemination of knowledge to the wider audience about the importance of agri-food science; one of the most important areas of research strategies in Europe and beyond.

AGROSYM 2017 was made possible through the commitment and contributions of a wide range of partners and sponsors. I take this opportunity to thank all of them and I look forward to a successful joint organization of AGROSYM 2018.

East Sarajevo, 23rd October 2017

Prof. Dušan Kovačević, PhD
Editor in Chief

CONTENT

IMPACT OF SLAVOL MICROBIOLOGIAL FERTILIZER ON SOIL MICROORGANISMS DURING CAULIFLOWER (*BRASSICA OLERACEA* L.*VAR. BOTRYTIS*) GROWTH

Daniela DIMOVSKA[1]*, Zvezda BOGEVSKA[1], Igor ILJOVSKI[1], Maria ZDRAVKOVSKA[1], Dzoko KUNGULOVSKI[2] , Natalia ATANASOVA- PANCHEVSKA[2]

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Abstract

The experiment was conducted in order to determine the influence of the microbiological fertilizer Slavol on the number of microorganisms in the soil where cauliflower was grown in open field.
It was used the variety Barselona F1 which was grown in Skopje region during three years (2011, 2012, 2013). The treatments were as follows: Ø control - without use of microbiological fertilizer, V-1 - foliar treatment every 7 days with 0.1% solution of Slavol and V-2 - drip irrigation treatment every 2 days with 0,1% solution of Slavol. The total number of bacteria and the number of examined physiological groups of microorganisms in the rhizosphere (nitrogen fixing bacteria 10^{-4}, cellulolytic microorganisms 10^{-4}, yeasts 10^{-4}, nitrifying microorganisms 10^{-4} and molds 10^{-3}) were counted.
According to the results during three years examination highest average number of total bacteria was determined in the variant V-1 and V-2 in comparison to control and the soil before planting. The number of nitrogen fixing bacteria was from 4863519 in V-1 to 4923807 in V-2. The number of cellulolytic microorganisms was from 3288588 in V-1 to 3312114 in V-2. The number of yeasts was lower than control (3813208) and was from 3681506 in V-1 to 2585089 in V-2. The number of nitrifying microorganisms was very high in V-1 (7502534) and V-2 (7323212) in comparison to control (1331717) and fallow land. The number of molds was higher in V-1 (422192) and V-2 (352608) in comparison to control (340149) but lower in comparison to fallow land (474851).

Keywords: *cauliflower, microbiological fertilizer, groups of microorganisms, rhizosphere*

Introduction

The activity of microorganisms in agro-ecological systems is influenced by the physical-chemical properties of soil, climatic conditions, agro-technical measures, plant species, pesticide and heavy metals content (Milosevic et al., 1997; Jarak et al., 2003).
In the production of some vegetable crops which produce relatively high yields, intensive soil cultivation, different agro-technical measures, large amounts of mineral fertilizers and pesticides are used which contributes to disturbance of balance between microorganisms and soil, soil degradation and overall disruption of the course of natural processes.
In our study, cauliflower (*Brassica oleraca var. botrytis*) was used which belongs to *Brassicaceae* L. family. It is characterized by high nutritional value and medicinal properties due to its chemical composition. Cauliflower is rich in many vitamins, minerals, proteins, carbohydrates and fats. The cauliflower is characterized by a pleasant and refreshing taste and it can be consumed freshly prepared in a different way or processed. Thus it is appreciated in households and industry throughout the year.
For high and qualitative yields in the cauliflower, it is necessary to fertilize with a combination of organic and mineral fertilizers Simonov and Aladzajkov (1985).

In order to improve the microbiological activity in the rhizosphere, today by improving the isolation method and obtaining pure cultures of microorganisms there are microbiological fertilizers – bio-fertilizers that treat crops with different methods of application (with a sprinkler or drip system). In order to improve growing conditions and obtain greater and better yields of cauliflower, microbiological fertilizer Slavol (combination of 6 bacteria, natural vitamins, enzymes and bio stimulators) was used. The objective of the research was to count the number of microorganisms in the soil treated with the microbiological fertilizer Slavol in cauliflower.

Materials and methods

The experiment was conducted in the vicinity of Skopsko, the Jurumleri settlement with GPS coordinates 41 ° 58'20.84" north and 21 ° 33'24.44" east of 276m altitude, on the soil type alluvium, during 2011, 2012 and 2013.

The subject of the trial was cauliflower (*Brassica oleracea L. var. botrytis*), hybrid Barcelona F_1, which was grown by seedlings in cold beds, and planted in the open field.

During 2011 and 2012, the sowing was carried out on May 20, and the planting was on July 16, while in 2013 the sowing was on May 25, and the planting was on July 17.

The trial was designed according to randomized complete block design according to the Fisher Method in three treatments, with four repetitions.

The treatments were based on the time and method of treatment with the microbiological fertilizer, Slavol, which is a combination of 6 bacteria belonging to the group of free nitrogen fixing bacteria *Azotobacter chrococcum* (108 cfu / ml) *Azotobacter vinelandii* (108 cfu / ml), *Derxia sp.* (109 cfu / mL), and phosphorous decomposing bacteria *Bacillus licheniformis* (109 cfu / mL), *Bacillus suptilis* (109 cfu / mL), *Bacillus megaterium* (109 cfu / mL). It also contains natural vitamins, enzymes and bio-stimulators.

The treatments were according following order:

1. Ø control - without the use of microbiological fertilizer;
2. Variant 1 (V-1) – tretating the seedlings by immersion in a solution of 5 L water and 50 mL Slavol for 5 minutes and treating during vegetation through leaves with portable sprayer every 7 days with a solution of 2 mL Slavol dissolved in 2 L water and
3. Variant 2 (V-2) - treating the seedlings by immersion in a solution of 5 L water and 50 mL Slavol for 5 minutes and treating during vegetation through drip system (spaghetti type) with a solution of 150 mL Slavol dissolved in 150 L water with irrigation rate per emitter of 2 L per hour, every two days.

Also, at the end of the vegetation before harvest, three samples from the rhizosphere of the soil with cauliflower were taken for microbiological analyzes (∅, V-1, V-2).

The following groups of microorganisms were examined before the beginning and the end of the vegetation: total number of bacteria in soil (Sarić, 1992), nitrogen fixing bacteria (Jarak, 2004), nitrifying microorganisms (Jarak, 2004), cellulolytic microorganisms (Sarić, 1992), yeasts (Govedarica et al.,1997) and molds (Govedarica et al.,1997).

Table 1 shows the composition of the growth medium for the examined groups of microorganisms.

Table 1. Examined groups of microorganisms, growth medium and incubation period

	Growth medium	**Composition of growth medium**	**Incubation period**
Total number of bacteria	Agar plus nutrients	agar 15 grL^{-1}, peptone 15 grL^{-1}, beef extract 3 grL^{-1}, NaCl 5 grL^{-1}, K_2HPO_4 0,3 grL^{-1}	5 days on 28 °C
Groups of microorganisms			
Nitrogen fixing bacteria	esbi agar	sucrose 20 grL^{-1}, K_2HPO_4 0,2 grL^{-1}, $MgSO_4$ x $7H_2O$ 0,2 grL^{-1}, K_2SO_4 0,1 grL^{-1}, $CaCO_3$ 5 grL^{-1}, agar 15 grL^{-1}	7 days on 28 °C
Nitrifying bacteria	Mineral medium	$(NH4)_2SO_4$ 2 grL^{-1}, K_2HPO_4 1grL^{-1}, $MgSO_4$ 0,5 grL^{-1}, Fe SO_4 0,4 grL^{-1}, NaCl 0,4 grL^{-1}, $CaCO_3$ 1grL^{-1}, Mg CO_3 1grL^{-1}, agar 15grL^{-1}, distilled water 1 L	5-7 days on 22 ^{0}C
Cellulolytic bacteria	Waxman –Garey	$(NH_4)_2$ HPO_4 2.5 grL^{-1}, $MgSO_4$ 0,5 grL^{-1}, $FeSO_4$ 0,01 grL^{-1}, KCl 0.5 grL^{-1}, $CaCl_2$ 0.02 grL^{-1}, $MnSO_4$ 0.001 grL^{-1}, agar 15 grL^{-1}, Na-carboxy methyl cellulose2grL^{-1}	7-14 days on 28 °C
Yeasts	Czapek-Dox Agar	$NaNO_3$ 2 g/L, KH_2PO_4 1grL^{-1}, $MgSO_4$ 0,5 grL^{-1}, KCl 0,5 grL^{-1}, $FeSO_4$ 0,01 grL^{-1}, sucrose 30 grL^{-1}, agar 20 grL^{-1}	7 days on 25 °C
Molds	Czapek-Dox Agar	$NaNO_3$ 2 grL^{-1}, KH_2PO_4 1 grL^{-1}, $MgSO_4$ 0,5grL^{-1}, KCl 0,5 grL^{-1}, $FeSO_4$ 0,01 grL^{-1}, sucrose 30 grL^{-1}, agar 20 grL^{-1}	7 days on 25 °C

After the incubation, the number of microorganisms brought up in the petri plates was determined. The total number of bacteria for all physiological groups of microorganisms on gram absolutely dry soil is calculated empirically according to the formula (Sarić, 1992; Govedarica et al., 1997):

$$x = \frac{a \times b \times c}{d}$$

a= average number of grown colonies;
b= amount of inoculum;
c= dilution;
d= mass of one g absolutely dry soil.

The obtained results were the subject of further analysis through the arithmetic mean, the ratio for calculating and representing the number of bacteria by variants compared to the number in fallow land and control.

Results and discussion

Table 2 shows the number of all examined groups of microorganisms in order to be perceived the influence of the applied microbiological fertilizers.

Table 2. Impact of the microbiological fertilizer on soil microorganisms status

	Number of microorganisms (thousands)							
	Treatment				Treatment			
	Fallow land	Control	V-1	V-2	Fallow land	Control	V-1	V-2
	Total number of bacteria				**N-fixing bacteria**			
2011	2 047.6	2 341.5	7 364.7	12 452.4	602.38	209.76	131.65	3 561.90
2012	2 295.4	7 090. 9	8 439.0	6 3230.0	3 529.55	2 241.56	3 665.85	3 595.06
2013	2 795.1	35 853.7	25 011.8	26 891.6	1 397.56	10731.71	9 607.06	7 614.46
Average	2 379.4	15 095.3	13 605.2	15 221.6	1 843.16	4 394 .34	4 863.52	4 923.81
IFL	100	634,4	571.7	639.7	100	238.4	263.8	267.1
IC		100	90.1	100.8		100	110.6	112.0
	Cellulolytic bacteria				**Yeasts**			
2011	595.24	763.41	3 065.88	3 014.29	809.52	600.00	2 423.53	1 157.14
2012	3 763.64	812.99	2 463.41	3 365.43	1 802.27	1 929.87	2 748 05	2 222.22
2013	682.93	5 860.98	4 336.47	3 556.63	107.50	8 909.76	5 872.94	4 375.90
Average	1 680.60	2 479.13	3 288. 9	3 312.1	906.43	813.21	3 681.51	2 585.09
IFL	100	147.5	195.6	197.0	100	420.6	406.1	285.1
IC		100	132.6	133.6		100	96.5	67.7
	Nitrifying bacteria				**Molds**			
2011	38. 10	397. 6	2 305.88	1 292.86	809.52	156.10	829.65	607.14
2012	533.18	1 280.52	2 648.78	3 086.42	604.54	3.38	6.34	36.05
2013	782.50	2 317.07	17552.94	17590.36	10.49	860. 98	430.59	414.63
Average	451.26	1 331.72	7 502.53	7 323.21	474.85	340.15	422. 19	352. 61
IFL	100	295.1	1662.5	1622.8	100	71.6	88.9	74.2
IC		100	563.3	549.9		100	124.1	103.6
***** IFL = index of fallow land; IC = Index of the control								

The total number of soil bacteria is considered the main indicator of its liveliness. The total number of microorganisms is the total number of bacteria that grow on soil agar (Govedarica et al., 1999). Depending on the type of soil, the total number of bacteria ranges from several hundred thousand to hundreds million in gram-soil (Govedarica and Jarak, 1995). In the three years of examination, the total number of bacteria has increased significantly in all variants at the end of vegetation in relation to fallow land. Regarding the index control, it can be concluded that bacteria have only slightly increased in V-2 by 0.8% in terms of control, while the number of bacteria in V-1 decreased by 9.9% in comparison to control.Trials on the total number of bacteria in different species showed that in sugar beet (Govedarica et al., 1999), potato (Najdenovska et al., 2004), soybean and corn (Jarak et al., 2004), the largest number of bacteria was at the end of vegetation. For the life and activity of the free aerobic nitrogen fixing bacteria, as well as for the nitrogen fixation of great importance are some of environmental factors and influences. Thus, if the aeration of the soil is greater, the faster the development of nitrogen fixing bacteria is, and the nitrogen elevation is also higher. Soil moisture also plays a significant role, and it has been established that nitrogen fixation occurs in soils with a moisture of 3 to 25% and more. The reaction of soil and soil temperature also influence the process of nitrogen fixation, where nitrogen fixation occurs in acid soils poorly or not at all, while in soils with neutral to weak alkaline reaction, or pH from 7 to 8, nitrogen fixation takes place more intensively. The temperature range in which nitrogen fixation takes place is from 7 to 35 ° C (Micev et al., 1988). Nitrogen fixing bacteria are an important component of rhizosphere microorganisms (Malik et al., 2005), for which Mrkovač et al. (2006) indicate that they are entering into community with non-leguminous crops, which is important for sustainable agricultural production. Nitrogen fixation is of great importance in the process of maintaining the nitrogen balance in the soil, and in general the fertility of the soil. In our study, the soil has neutral to

moderate alkaline reaction. It is structural with a good ratio of capillary and non-capillary pores, which is a convenient environment for the development of nitrogen fixing bacteria. On average for the three years of examination, nitrogen fixing bacteria have increased in all variants during vegetation in relation to fallow land. The highest increase was obtained in V-2 (167.1%), then in V-1 (163.8%), while in the control the increase was 138.4%. Regarding the index to control between variants at the end of vegetation, a greater presence of nitrogen fixing bacteria was found for 10, 6% in V-1 and 12% in V-2. From the foregoing we can conclude that the variants treated with microbiological fertilizer have a higher presence of nitrogen fixing bacteria in comparison to untreated variant - control. From previous investigations regarding the presence of nitrogen fixing bacteria, Govedarica (1986) found that in the corn the nitrogen fixing bacteria were most present in the rhizosphere at the end of the vegetation. Najdenovska et al. (2004) also found the greatest presence of azotobacter in the application of pure culture *Azotobacter chroococcum* at the end of vegetation in all three years studied in potato varieties latona and lizeta. Aerobic cellulose bacteria represent a separate physiological group that has adapted to using cellulose as a source of carbon and energy. In the three years of the study, the number of cellulolytic bacteria increased in all variants in relation to fallow land. The highest increase was recorded in V-2 (97.0%). Regarding the control index at the end of the vegetation, it was found that in V-1 the number of cellulolytic bacteria increased by 32.6%, while in V-2 it increased by 33.6% (Table 2). Yeasts in soil are present in smaller numbers and are important because they decompose monosaccharaides under anaerobic conditions. Yeasts are more common in soils with acid pH and high humidity such as peat soils. Table 2 gives data on the average number of yeasts per year in relation to the index of fallow land before vegetation and at the end of vegetation in control, V-1 and V-2. In the three years of the study, the number of yeasts increased in all variants in comparison to fallow land. The highest increase was obtained in the control variant (320.6%). The average number of yeasts for three years of testing in terms of control at the end of vegetation has been reduced in variants treated with microbiological fertilizer. The presence of nitrifying bacteria in the soil depends on the presence of free oxygen and the reaction of the soil. In soils with a pH below 5 the number of nitrifying bacteria is low. Nitrifying bacteria are widely represented in nature. They are found in soils with sufficient humidity and aeration, favorable pH and nutritional conditions. The optimum temperature for their activity is 30-35°C. According to the data presented in Table 2, in the three years of the study nitrifying bacteria have increased only in the first and second variants in vegetation depending on the prevalence before vegetation. Regarding the index at the end of vegetation, between control and variants, a significant increase in the number of nitrifying bacteria was observed in the variants treated with microbiological fertilizers in comparison to control.

The molds are multinuclear, filamentous, bonded, aerobic fungi, built of hyphae. The optimal conditions for the development of the mold are a temperature of about 30°C, moisture, pH 6 and free access to oxygen (Sarić, 1991). In the three years of examination in relation to the soil that is not sown (fallow land), the mold decreased in control, V-1 and V-2. Regarding the index control at the end of vegetation, between control and variants, it was determined that V-1 and V-2 had a higher number of mold compared to control (24,1% and 3,6% respectively).

Conclusion

In our study the highest values of total number of bacteria and nitrogen fixing bacteria were found in the V-2 treatment, the presence of the yeast is the highest in control, while under fallow land conditions were found the highest presence of molds. The average number of cellulolytic bacteria is highest in V-2, and the insignificant difference in relation to V-2 is also obtained in V-1. The average number of nitrifying bacteria is greatest in variants treated with microbiological fertilizer V-1 and V-2.

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