

SOIL MICROBIAL ACTIVITY AS INFLUENCED BY INTEGRATED
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Abstract: Over the two-year study (2008–2009) we monitored the influence of integrated and conventional production systems on microbiological activity in soil and strawberry yield. The experiment also involved fertilizers applied in three variants of treatment. The studied parameters were monitored over 2008 and 2009 by determining the total number of soil microorganisms, the number of ammonifying bacteria and the strawberry yield. The results of the study suggest the pronounced inhibitory effect of insecticides on number of studied microorganism groups in all three conventionally treated variants, over the both years of study, which further infers negligible stimulative influence of strawberry plants on yield.

INTRODUCTION

Extensive agricultural activities over the past six decades and increasing utilization of synthetic nitrogen fertilizers have resulted in excessive environmental pollution. Aiming at high and quality yields, modern agricultural production, glasshouse production in particular, necessitates the application of pesticides [1, 3]. In developed countries, 60% of fruit production is based on plant protection chemicals [5]. However, the application of these chemicals can be accompanied by a series of undesirable effects on other members of biocoenosis, primarily on soil microbial community, which within the food chain plays a role in their transfer towards higher life forms and man [6].

Cultural practices that are applied today in fertilization [7] and for plant diseases control, suggest the possibility of using some alternative methods, as well as biological agents. Despite the fact that utilization of such agricultural production system could provide steady and environmentally friendly yields, this production framework cannot be said to be applied at the expected pace. The decreased production, as a consequence of the reduced application of both mineral fertilizers and protection chemicals, accounts for current state [11]. However, according to the same author, financial returns gained by the integrated production system are steady owing to higher prices of products obtained in this manner.

Modern plant production imposes high profitability requirements, however high requirements in terms of preservation of environment presuppose a careful approach to nutrition and protection of agricultural crops. Therefore, the objective of this study was to investigate the influence of integrated and conventional production systems on the total number of soil microorganisms and ammonifying bacteria, and the yield accordingly.

MATERIAL AND METHODS

The trial was set up in glasshouse of Fruit Research Institute, Cacak (Serbia) in the autumn 2007 and it included strawberry plants planted in a randomized block design in three replications. Cv. Senga Sengana, planted in 10 dm³ growing vessels, was used as the test plant. Two production systems were employed (factor A) – integrated (A₁) and conventional (A₂). The former system involved the application of biological insecticides, i.e. neem (NeemAzal 0.4%) and pyrethrin (Pyros 0.2%), whereas the latter included chemical insecticides, i.e. endosulphan (Thiosulphan 0.2%) and gusathion (Gusathion 0.15%). Both insecticide categories were applied in two replications, in both years of study.

The experiment also involved three fertilizer variants of treatment (factor B), i.e. B₁ – inoculum of nitrogen-fixing bacteria, species *Klebsiella* (the working title of microbiological fertilizer Enteroplantin), B₂ – microbiological fertilizer Slavol; B₃ – highly soluble mineral fertilizer Multi KMg and Control – non-fertilized soil.

Microbiological fertilizer Enteroplantin is the pure culture of *Gram*-negative nitrogen fixing bacteria *Klebsiella planticola* obtained from the collection of microorganisms of Microbiology Laboratory, Faculty of Agronomy, Cacak (Serbia). The bacteria titre ranged from 20 to 40 x 10⁶.cm⁻³. Microbiological fertilizer Slavol is made up of the combined nitrogen fixing and phosphorus mineralizing bacteria (*Azotobacter chroococcum*, *A. vinelandi*, *Dexia* sp., *Bacillus megatherium*, *B. lichenformis* and *B. subtilis*), the production line of Agrounik DOO Company, Zemun (Serbia) [2]. The procedure first included the immersion of strawberry roots into the liquid inoculum of the mentioned bacteria whereupon plants were watered for four months with this inoculum (100 cm³) during growing period in both 2008 and 2009. 12:0:43 + 2MgO Multi KMg is a water-soluble mineral fertilizer (production line of ‘Haifa Chemicals Ltd’- Israel) [14]. It was mixed with 100 ml water (per planting vessel) for four months during growing period (2008–2009) on short-term basis (0.8 g fertilizer/500 ml water). The control was also watered with the same quantity of water.

Klasmann TS1 standard, recommended for growing of salt-sensitive plants, was used as the growing medium. It is the mixture of white sphagnum peat, lime, water-soluble fertilizer (1 g.l⁻¹) and microelements. Its structure is conventional (0–25), of low-acid reaction (pH in H₂O = 6).

Strawberry plants were subjected to thermal processing before planting, i.e. they were immersed for 10 minutes into water heated up to 46°C.

Microbiological analyses of the soil were carried out in 2008 and 2009.

Soil samples for microbiological analyses were taken twice during the growing period (factor C). In both years of study, the first sampling (C₁) was done in the germination period (April, 10), the second (C₂) followed the phase of technological maturity of fruits (June 10). The total number of microorganisms and the number of ammonifying bacteria was checked by the dilution method (0.5 cm³ 10⁻⁶) on appropriate selective solid

media [8]. The total number of microorganisms was determined on soil agar, whereas the number of ammonifying bacteria was checked on meat-peptone agar. Additionally, in the determination of the number of the grown colonies, Nessler reagent was applied in order to verify the presence of ammonia around it. All the analyses were done in three replications, and the mean number of colonies was projected into soil dry mater [13] [CFU·g⁻¹ d.m. soil].

Strawberry yield was determined in the phase of technological maturity, and has been expressed as g·strawberry runner⁻¹.

The data obtained in this study were subjected to analysis of variance method [12] of trifactorial 2 x 4 x 2 trial form (production system x applied fertilizers x sampling period) for microbiological analyses, and 2 x 4 x 2 trial form (production system x applied fertilizers x year of study) for strawberry yield. Significance of differences in individual and interaction means was tested by lsd test.

RESULTS AND DISCUSSION

Analysis of variance of the total number of microorganisms suggests statistically high influence of the factors A (fertilizer) and B (production system). Similarly, the interaction effect of A x B (fertilizer x production system) also infers high statistical significance.

The obtained results for the total number of soil microorganisms (Tabs. 1, 2) suggest a pronounced effect of insecticides applied within the conventional production system (Endosulphan and Gusathion) in both years of study. In line with the results above, Sohail and Muhammad revealed the decline in number of soil microorganisms which resulted from the application of 1000 ppm of Chlorpyrifos and Endosulphan [10].

Table 1. Total number of microorganisms (CFU·10⁶·g⁻¹ d.m. soil) as affected by the production system (A), applied fertilizer (B) and sampling period (C) in 2008

Fertilizer (B)		B ₁	B ₂	B ₃	Control	\bar{X}		
Protection system (A)	A ₁	Period (C)	C ₁	36.667	31.333	11.333	10.000	23.250
			C ₂	42.667	34.667	9.667	9.667	
	\bar{X}		39.667	33.000	10.500	9.833		
	A ₂	Period (C)	C ₁	12.333	10.000	9.333	2.667	8.167
C ₂			11.000	8.000	6.000	6.000		
\bar{X}		11.667	9.000	7.667	4.333			
\bar{X}_B		25.667	21.000	9.083	7.083	15.708		
\bar{X}_C		C ₁	24.500	20.667	10.333	6.333	15.458	
		C ₂	26.833	21.333	7.833	7.833	15.958	
lsd								
lsd		A	B	C	AxB	AxC	BxC	AxBxC
0.05		5.36	3.79	3.79	7.59	7.59	5.36	10.73
0.01		7.21	5.10	5.10	10.20	10.20	7.21	14.42

Table 2. Total number of microorganisms (CFU·10⁶·g⁻¹ d.m. soil) as affected by the production system (A), applied fertilizer (B) and sampling period (C) in 2009

Fertilizer (B)			B ₁	B ₂	B ₃	Control	\bar{X}		
Protection system (A)	A ₁	Period (C)	C ₁	58.000	41.667	30.000	19.667	40.083	
			C ₂	70.000	57.333	23.000	21.000		
	\bar{X}			64.000	49.500	26.500	20.333		
	A ₂	Period (C)	C ₁	14.667	13.667	12.667	6.333	11.708	
			C ₂	17.333	17.333	6.000	5.667		
		\bar{X}			16.000	15.500	9.333		6.000
\bar{X}_B			40.000	32.500	17.917	13.167	25.932		
\bar{X}_C			C ₁	36.333	27.667	21.333	13.000	24.584	
			C ₂	43.670	37.333	14.500	13.333	27.208	
lsd									
lsd			A	B	C	AxB	AxC	BxC	AxBxC
0.05			8.22	5.81	5.81	11.62	11.62	8.22	16.44
0.01			11.05	7.81	7.81	15.62	15.62	11.05	22.10

Govedarica *et al.* reached similar results by studying the influence of Miral 500 GS, Cimogal and Acetilica 50 EC on soil microbiological activity [4]. The investigation of the effect of Dimethoat and Fenitrothionin soil in an apple nursery revealed the inhibitory influence of insecticides on the development of amylolytic microorganisms and *Azotobacter* [6].

The inhibitory effect of Endosulphan and Gusathion on the total number of soil microorganisms over both years of study was particularly pronounced in variants of treatment with Enteroplantin and Slavol (B₁ and B₂), and in 2009 the effect was also observed in the variant of treatment which included mineral fertilizer (B₃).

Experimental results of the influence of conventional and integrated production systems on number of ammonifying bacteria, treated with mineral and microbiological fertilizers and monitored twice in each year of study suggested highly significant statistical effect of all the studied factors (A, B, C) and their interactions during 2008 and 2009 (Table 3, 4).

The interaction of production systems and applied fertilizers (A x B) and their influence on number of ammonifying bacteria point to their substantially lower number in conventional production system method, which is the result of the toxic effect of chemical insecticides applied within this production system. The intensity of the inhibitory effect of Endosulphan and Gusathion on development of soil ammonifying bacteria varied, and it was most pronounced (statistically highly significant) in the variant of treatment with Enteroplantin (B₁) during 2008, whereas in the following year this effect was evidenced in the variants of treatment which included Slavol (B₂) and Multi KMg (B₃).

As for growing period (A x C), a more pronounced toxic effect of Endosulphan and Gusathion on the studied biological parameters of soil was evidenced in the first period (April, 10) in both years, in all the variants of treatment, except in the one with mineral fertilizer. Such results are quite expected given that soil samples intended for analysis were taken 8 and 10 days (2008 and 2009 respectively) after treatment. The study of the im-

Table 3. The number of ammonifying bacteria (CFU·10⁶·g⁻¹ d.m. soil) as affected by the production system (A), applied fertilizer (B) and sampling period (C) in 2008

Fertilizer (B)			B ₁	B ₂	B ₃	Control	\bar{X}		
Protection system (A)	A ₁	Period (C)	C ₁	19.667	13.000	12.667	11.000	18.375	
			C ₂	53.333	18.000	9.33	10.000		
	\bar{X}			36.500	15.000	11.000	10.500	8.167	
	A ₂	Period (C)	C ₁	14.667	10.667	6.333	4.333		
C ₂			7.667	9.000	5.667	7.000			
\bar{X}			11.167	9.833	6.000	5.667			
\bar{X}_B			23.833	12.667	8.500	8.083	13.271		
\bar{X}_C			C ₁	17.167	11.833	9.500	7.667	11.542	
			C ₂	30.500	13.500	7.500	8.500	15.000	
lsd									
lsd			A	B	C	AxB	AxC	BxC	AxBxC
0.05			4.02	2.84	2.84	5.69	5.69	4.02	8.04
0.01			5.41	3.82	3.82	7.64	7.64	5.41	10.81

Table 4. The number of ammonifying bacteria (CFU·10⁶·g⁻¹ d.m. soil) as affected by the production system (A), applied fertilizer (B) and sampling period (C) in 2009

Fertilizer (B)			B ₁	B ₂	B ₃	Control	\bar{X}		
Protection system (A)	A ₁	Period (C)	C ₁	39.000	27.667	30.000	18.667	33.208	
			C ₂	52.667	41.333	34.333	22.000		
	\bar{X}			45.834	34.500	32.166	20.333	7.542	
	A ₂	Period (C)	C ₁	14.333	12.667	10.333	3.000		
C ₂			6.667	5.333	4.667	3.333			
\bar{X}			10.500	9.000	7.500	3.167			
\bar{X}_B			28.167	21.750	19.833	11.750	20.375		
\bar{X}_C			C ₁	26.667	20.167	20.167	10.833	19.458	
			C ₂	29.667	23.333	19.500	12.667	21.292	
lsd									
lsd			A	B	C	AxB	AxC	BxC	AxBxC
0.05			8.13	5.75	5.75	11.50	11.50	8.13	16.26
0.01			10.93	7.73	7.73	15.45	15.45	10.93	21.85

fact of Dursban and Zinophos on microbial activity of soil has revealed that their most pronounced negative effect was observed on the seventh day after application, whereas it was fully eliminated after 4–6 weeks [13], as confirmed by our results [9]. Similar results were evidenced in the investigation into the effect of Cypermthrin and Imidacloprid [10].

Experimental data on the effect of applied fertilizer (A), production system (B) and year of study (C) on strawberry yield are shown in Table 5.

Table 5. The average strawberry yield (g:strawberry runner⁻¹) as affected by the applied production system (A), applied fertilizer (B) and research year (C)

Fertilizer (B)			B ₁	B ₂	B ₃	Control	\bar{X}		
Protection (A)	A ₁	Year (C)	C ₁	437.197	386.850	400.467	320.563	426.023	
			C ₂	557.170	558.387	451.580	427.400		
	\bar{X}			507.183	472.618	426.023	373.982		
	A ₂	Year (C)	C ₁	507.030	487.323	640.480	420.260	531.510	
			C ₂	584.540	557.710	573.440	481.300		
		\bar{X}			545.785	522.517	606.960		450.780
\bar{X}_B			526.484	497.568	516.492	412.381	488.231		
\bar{X}_C			C ₁	472.113	437.087	520.473	370.412	450.021	
			C ₂	580.855	558.048	512.510	454.350	526.441	
lsd									
lsd			A	B	C	AxB	AxC	BxC	AxBxC
0.05			136.06	96.21	96.21	192.42	192.42	136.06	272.12
0.01			182.91	129.34	129.34	258.67	258.67	182.91	365.82

Statistical analysis suggests that the above factors did not influence significantly strawberry yield. Negligibly higher yield was evidenced in conventionally grown plants as well as in the variant of treatment which included microbiological fertilizer Enteroplantin.

CONCLUSIONS

1. Application of endosulphan (Thiosulphan 0.2%) and Gusathion (Gusathion 0.15%), as a measure of conventional protection of strawberry plants has brought about the decline in the total number of microorganisms and the number of ammonifying bacteria;
2. Application of neem (NeemAzal 0.4%) and pyrethrin (Pyros 0.2%), as a measure of integrated protection of strawberry plants has resulted in the increase in the total number of microorganisms and the number of ammonifying bacteria;
3. In both years of study, variants of treatment which included Enteroplantin and Slavol had a stimulating effect on biological parameters of soil;
4. The effect of the applied production systems were more pronounced in the second sampling period;
5. The applied production systems, fertilizers and years of study did not significantly influence strawberry yield; negligibly higher yield was evidenced in conventionally grown plants which utilized microbiological Enteroplantin.

Given the biological characteristics of soil and the yield, as well as all the advantages in respect of health status of strawberry fruits, we have concluded that the application of integrated production system can be recommended for the glasshouse production of strawberry.

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AKTYWNOŚĆ DROBNOUSTROJÓW GLEBOWYCH POD WPLYWEM ZINTEGROWANYCH I KONWENCJONALNYCH SYSTEMÓW PRODUKCJI

Podczas dwuletnich badań (2008–2009) sprawdzono wpływ zintegrowanych i konwencjonalnych systemów produkcji na aktywność mikrobiologiczną w glebie oraz ich wpływ na plony truskawek. Eksperyment obejmował stosowanie nawozów w trzech różnych wariantach leczenia. Podczas badań określono (w latach 2008–2009) ogólną liczbę mikroorganizmów glebowych, liczbę bakterii amonifikacyjnych i plon truskawek. Wyniki sugerują ważny efekt hamujący insektycydów na liczebność badanych grup mikroorganizmów we wszystkich trzech wariantach stosowanych konwencjonalnych procedur w ciągu dwóch lat badań, co dowodzi nieistotnego stymulującego wpływu na uprawy truskawek.