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EFFECT OF CONVENTIONAL AND BOTANICAL INSECTICIDES ON SOIL MICROBIAL ACTIVITY

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Abstract. The laboratory and field studies were conducted to determine the effect of the application of conventional and botanical insecticides on microbiological activity in the soil. The trial was set up in the autumn 2007 in glasshouse of Fruit Research Institute, Cacak, Republic of Serbia. In vegetative 10-litre vessels, strawberry runners, immersed for 10 min in water heated to 46°C, were planted. The trial included 4 treatment variants (I – microbiological fertiliser Enteroplantin; II – microbiological fertiliser Slavol; III – mineral fertiliser Multi KMg; IV – non-fertilised soil) as well as the application of conventional and botanical insecticides. The experiment was carried out under the randomised block design system, in 3 replications. Over the growing season, conventional measures involved the application of endosulphan (Thiosulfan 0.2%) and gusathion (Gusathion 0.15%). As for botanical insecticides, neem (NeemAzal 0.4%) and pyrethrin (Pyros 0.2%) were used as the part of organic production system. The effect of the applied insecticides was determined twice over the growing season and was monitored by checking the fungal and actinomycetes count. Indirect dilution methods of growing on adequate culture media were used to determine the number of studied microorganisms groups. The two-year results (2008–2009) suggest that the insecticides applied within conventional production system caused significant reduction in the number of the soil fungi.

Keywords: insecticides, microorganisms, soil, botanical, conventional insecticides.

AIMS AND BACKGROUND

Plant growing under protected conditions has been taking on a new significance, since such conditions and the application of adequate technology ensure a continuous plant production[1]. However, the above conditions favour the development of numerous damaging organisms, e.g. insects, mites, nematodes, fungi, bacteria, viruses, phytoplasms, etc. which impede or seriously hamper plant growing. Aiming at high, quality yields, modern agricultural production necessitates the application

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of pesticides[2,3], which deeply influences both human health and environment[4]. An increasing amount of data suggest that trace pesticide can contaminate surface and underground waters and cause undesirable effects not only on cultivated plants but also on beneficial soil organisms. Additionally, pesticide residues can also be found in food. Soil microbial community also suffers the consequences of pesticides application which vary among types, concentrations, soil types, applied cultural practices, and can be accompanied by a wide range of phenomena – from the stimulation, past repression, and all through to the occurrence of resistant mutant soil organisms species or even microorganisms that effectively decompose pesticides allowing for the soil autopurification[5,6]. The latest research infer possible utilisation of some alternative methods as well as the effective application of biological agents intended for the protection of plants from different causal agents of diseases[7].

Given that soil microorganisms, seen as a biogeocenosis component, are an important object of pesticides activity and considering their participation in food chain towards higher organisms and humans, we have determined the objective of this investigation – the effect of conventional and integrated production on the number of soil fungi and actinomycetes in strawberry growing under glasshouse conditions.

EXPERIMENTAL

The trial was set up in the autumn 2007 in glasshouse of Fruit Research Institute, Cacak. The experiment was set up under the randomised block design system, in 3 replications. Cultivar 'Senga Sengana', planted in 10-litre vessels, was used as the test plant. Integrated (A_1) and conventional (A_2) production systems were applied. During growing season, integrated system involved the application of neem (NeemAzal 0.4%) and pyrethrin (Pyros 0.2%), whereas conventional measures included endosulphan (Thiosulfan 0.2%) and gusathion (Gusathion 0.15%). The trial encompassed the following treatments: B_1 – inoculum *Klebsiella* sp. (work title – microbiological fertiliser Enteroplantin); B_2 – microbiological fertiliser Slavol; B_3 – highly soluble mineral fertiliser Multi KMg; B_4 – non-fertilised soil. Before planting, strawberry runners were subjected to thermal processing, and root inoculation by microbiological fertilisers Enteroplantin and Slavol. During growing season, the fertilisation program involved regular dressings with microbiological and mineral fertilisers.

The effect of the applied insecticides was determined twice over the strawberry growing season (C). The first sampling (C_1) was conducted during the fruit setting stage and the second (C_2) during the fruit maturity stage. The effect of the conventional and botanical insecticides was monitored by checking the fungal count (indirect method of dilution of 0.5 cm^3 10^{-5} on the Czapek agar) and actinomycetes count (indirect method of dilution of 0.5 cm^3 10^{-5} on the Krasilnikov synthetic agar)[8].

The data obtained from the microbiological analysis were subjected to the 3-factorial analysis of variance[9] in a 2 × 4 × 2 arrangement (production systems × fertiliser × sampling period), e.g. 2 × 4 × 2 arrangement (production systems × fertiliser × research year) from strawberry yield. The testing of the significance of differences between individual and interactive means was done using the Least Significant Difference (LSD)-test.

RESULTS AND DISCUSSION

The obtained results of the investigation into the effect of applied production systems, fertiliser types and sampling periods suggest that applied production systems and fertiliser types had a highly significant influence on the development of soil fungi over the entire research period, whereas sampling periods had such an effect only in season 2009.

The research results demonstrate that the insecticides applied within the conventional production system (endosulphan and gushation) had significant toxic effect on the development of soil fungi. Namely, conventional production system gave significantly smaller number of soil fungi in 2008 as compared to that obtained under the integrated production system (Table 1). This trend continued in the ensuing year resulting in highly significant number of soil fungi under the former system (6.292×10^5/1.0 g of absolutely dry soil), whereas the number of soil fungi in the latter system was 18.042×10^5 1.0 g of absolutely dry soil (Table 2). Similarly, an investigation of 5 insecticides on microbial and enzymatic activities showed inhibitory effect on imidacloprid on fungal numbers[10].

Table 1. Mean fungal count (10^5/1.0 g of absolutely dry soil) in soil as affected by the applied production system (A), fertiliser (B) and sampling period (C) in 2008

	Fertiliser (B)			B_1	B_2	B_3	Control	$\bar{x}$
Protection (A)	A_1	period (C)	C_1	7.667	8.333	4.333	2.333	4.958
			C_2	2.000	3.667	9.667	1.667	
		$\bar{x}$		4.833	6.000	7.000	2.000	
	A_2	period (C)	C_1	2.000	4.000	1.667	1.333	2.083
			C_2	1.667	1.333	3.333	1.333	
		$\bar{x}$		1.833	2.666	2.500	1.333	
		$\bar{x}_B$		3.333	4.333	4.750	1.666	3.521
	$\bar{x}_C$		C_1	4.833	6.166	3.000	1.833	3.958
			C_2	1.833	2.500	6.500	1.500	3.083

LSD

LSD	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.34	2.31	2.34	3.27	3.31	3.27	4.63
0.01	4.23	3.56	4.23	5.03	5.99	5.03	7.12

Table 2. Mean fungal count (10^5/1.0 g of absolutely dry soil) in soil as affected by the applied production system (A), fertiliser (B) and sampling period (C) in 2009

Fertiliser (B)				B_1	B_2	B_3	Control	$\bar{x}$
Protection (A)	A_1	period (C)	C_1	13.333	20.000	35.667	17.000	18.042
			C_2	14.667	18.667	18.000	7.000	
		$\bar{x}$		14.000	19.333	26.8333	12.000	
	A_2	period (C)	C_1	8.000	5.333	9.000	7.000	6.292
			C_2	6.000	5.000	7.333	2.667	
		$\bar{x}$		7.000	5.167	8.167	4.833	
		$\bar{x}_B$		10.500	12.250	17.500	8.417	12.167
	$\bar{x}_C$		C_1	10.667	12.667	22.333	12.000	14.417
			C_2	10.333	11.833	12.667	4.833	9.917

LSD

LSD	A	B	C	A×B	A×C	B×C	A×B×C
0.05	5.51	5.44	5.51	7.70	7.79	7.70	10.89
0.01	9.95	8.37	9.95	11.84	14.08	11.84	16.74

An investigation was carried out on 2 adjacent fields in State of Washington (the USA) whereby one of the fields was not treated either with mineral fertilisers and pesticides, and the other one included both mineral fertilisers and pesticides over a 30–35-year period. The comparison of the results showed that the former gave better results in terms of polysaccharides content, microbial biomass and activity of soil enzymes – urease, phosphase, hydrogenase[11].

In our trial, throughout the trial period, an exceptionally pronounced inhibitory effect was observed in treatments that involved microbiological fertilisers Slavol and Enteroplantin applied within conventional production system. During 2008, integrated production system revealed a highly pronounced stimulation in the treatment involving microbiological fertiliser Slavol and mineral fertiliser Multi KMg, whereas the same tendency was observed in 2009 in all treatments under the same production system.

From the seasonal aspect, the toxic effect of the above insecticides was more pronounced over the later trial period, i.e. statistically insignificant in season 2008 and even less significant in season 2009. Given that soil samples intended for the analysis were collected 10 days after being treated, the results were expected, which is in agreement with other authors results. The investigation of the effect of 4 organophosphorous insecticides on soil microbial activity revealed that the negative influence of the applied insecticides (Dursban, Zinophos) on the development of soil fungi was the most pronounced in the first week after the application and totally ceased 4–6 weeks after the application[12].

The development of soil actinomycetes was also governed by the employed production system, fertiliser type and sampling period, nevertheless the effect of the individual factors and the interactions was lower.

The analysis of the effect of production systems on the development of soil actinomycetes suggests statistically insignificant stimulating effect of the applied insecticides throughout the trial period. The number of soil actinomycetes during 2008 was insignificantly higher within conventional production system than within the contrasted system (Table 3). Similar results were recorded in 2009, i.e. 5.042 × 10^5/1.0 g of absolutely dry soil in conventionally grown plants, and 3.667 × 10^5/1.0 g of absolutely dry soil in plants grown under integrated production system (Table 4). This phenomenon suggests that highly developed enzyme system in actinomycetes enables them to disable even heavily degradable chemical compounds, e.g. pesticides, heavy metals, phenols, allowing them an undisturbed development even under the above conditions[13].

Table 3. Mean actinomycetes count (10^5/1.0 g of absolutely dry soil) in soil as affected by the applied production system (A), fertiliser (B) and sampling period (C) in 2008

	Fertiliser (B)			B_1	B_2	B_3	Control	$\bar{x}$
Protection (A)	A_1	period (C)	C_1	2.000	1.000	1.000	0.667	3.750
			C_2	14.667	7.333	2.000	1.333	
		$\bar{x}$		8.333	4.167	1.500	1.000	
	A_2	period (C)	C_1	5.333	2.000	1.667	1.000	4.792
			C_2	15.000	8.000	3.333	2.000	
		$\bar{x}$		10.167	5.000	2.500	1.500	
		$\bar{x}_B$		9.250	4.582	2.000	1.250	4.271
	$\bar{x}_C$		C_1	3.667	1.500	1.333	0.833	1.833
			C_2	14.833	7.667	2.667	1.667	6.708

LSD

LSD	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.78	1.76	1.78	2.48	2.51	2.48	3.51
0.01	3.21	2.70	3.21	3.82	4.54	3.82	5.40

Table 4. Mean actinomycetes count (10^5/1.0 g of absolutely dry soil) in soil as affected by the applied production system (A), fertiliser (B) and sampling period (C) in 2009

Fertilizer (B)				B_1	B_2	B_3	Control	$\bar{x}$
Protection (A)	A_1	period (C)	C_1	6.667	3.667	1.667	1.667	3.667
			C_2	6.000	4.667	3.000	2.000	
		$\bar{x}$		6.333	4.167	4.542	1.833	
	A_2	period (C)	C_1	7.000	5.667	4.000	3.000	5.042
			C_2	8.000	6.333	3.333	3.000	
		$\bar{x}$		7.500	4.167	3.667	3.000	
		$\bar{x}_B$		6.917	5.083	3.000	2.417	4.354
	$\bar{x}_C$		C_1	6.833	4.667	2.833	2.333	4.167
			C_2	7.000	5.500	3.167	2.500	4.542

LSD

LSD	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.92	2.89	2.92	4.09	4.14	4.09	5.78
0.01	5.28	4.44	5.28	6.29	7.47	6.29	8.89

Particularly pronounced stimulating effect on the development of soil actinomycetes was recorded in treatment variants that involved Enteroplantin and Slavol, under both production systems in 2008, whereas in the second year of trial (2009), highly significant stimulation was observed only in the treatment variant which included microbiological fertiliser Enteroplantin.

The number of actinomycetes was highly significantly higher over the second trial period of 2008, and was insignificantly higher over the same period of 2009.

The results of the performed study are in agreement with the results of the study of the effect of herbicides on soil microbiological activity. These implied the rise in the number of actinomycetes, and the decrease in the number of fungi under all applied herbicides rates and in all periods of sampling[14].

The experimental data on the effect of production system (A), fertiliser (B) and year of study (C) on strawberry yield are given in Table 5.

The statistical analysis of the obtained data showed no statistically significant differences in the factors tested or their interactions.

Overall, strawberry yield was insignificantly higher in the treatment involving use of the microbiological fertiliser Enteroplantin (B_1), as well as in plants grown under conventional system of production (A_2) and in the second year of the study (2009).

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

Fertiliser (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	A×B	A×C	B×C	A×B×C
0.05	196.11	193.80	196.11	274.08	277.34	274.08	387.60
0.01	354.41	298.05	354.41	421.51	501.21	421.51	596.11

CONCLUSIONS

The results of the study infer the following conclusions:

– the fungal and actinomycetes number were dependent on the applied production systems, fertiliser type and sampling period;

– the application of endosulphan (Thiosulfan 0.2%) and gusathion (Gusathion 0.15%) had an inhibitory effect on the development of soil fungi and a stimulating effect on soil actinomycetes;

– the application of neem (NeemAzal 0.4%) and pyrethrine (Pyros 0.2%) had a stimulating effect on the development of soil fungi and actinomycetes;

– during both years of study, the highest number of fungi and actinomycetes was recorded in treatment variants that included microbiological fertilisers – Enteroplantin and Slavol, in plants grown under integrated production system;

– the effect of the applied production systems was more pronounced over the second sampling period;

– the most significant increase in strawberry yield was observed with the use of microbiological fertiliser Enteroplantin (B_1), as well as in plants grown under conventional production system (A_2), in the second year of the study (2009).

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