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IMPACT OF SEED DRESSINGS ON MICROBIOLOGICAL ACTIVITY OF SOIL UNDER WINTER TRITICALE CULTIVATION

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Abstract: The aim of the presented investigations was to examine changes in the intensity of dehydrogenase and acid phosphatase activities as well as of the dynamics of selected groups of microorganisms in the soil under the cultivation of winter triticale following the application of the following seed dressings: (a.s.) flutriafol 2.5% + fludioxonil 2.5% in two doses and (a.s.) carboxin and tiuram. The experiment had a field character. The number of microorganisms (total bacteria, fungi, oligotrophic, copiotrophic and *Azotobacter*) was determined by the plate method on adequate agar substrates. Activity levels of the selected enzymes were defined using the spectrometrical method.

The obtained results indicate a change in the dehydrogenase and phoshatase activity in soil depending on the seed dressing applied in the experiment as well as at the date of investigations. The number of microorganisms in the soil underwent fluctuations depending on the developmental stage of triticale and the applied fungicide. The performed experiment demonstrated that counts of microorganisms in the soil underwent fluctuations depending on the developmental stage of triticale and the applied fungicide.

INTRODUCTION

Advancing anthropogenic pressure on the environment contributed to the contamination of soil with various toxic substances, including crop protection agents. Biocides can remain deposited in soil for several years, although majority of them exhibit limited stability in the soil environment. Pesticides introduced into the soil destroy not only harmful organisms but also beneficial elements of soil biosphere. On the other hand, soil microorganisms can decompose chemical preparations and weaken their action as a result of metabolic changes.

Bearing in mind the significance of this issue, it became necessary to determine the type and extent of disturbances appearing in the soil following application of pesticides.

The occurrence of biocides depends, to a considerable extent, on soil physico-chemical properties, a kind of the applied preparation as well as the kind and chemical structure of the employed active ingredient. Their excess exerts a negative impact on soil biological

activity indicated by enzymatic activity [1] and quantities of organisms living in the soil. Problems associated with soil enzymatic activity avoke growing interest among all researchers. It is possible to detoxificate of all kinds of contaminations finding their way into the soil as well as such substances as xenobiotics and pesticides, primarily, thanks to specific activity of microorganisms.

Enzymatic activity makes it possible to monitor the direction of metabolic transformations in soil taking place under the influence of plant protection agents [1]. According to Gliński *et al*. [5] and Koper, Siwik-Ziomek [10] soil enzymes as well as numbers of microorganisms are considered as an objective biological index of soil fertility, intensity of soil-forming processes and anthropo-pressure.

The aim of the presented investigations was to examine changes in the intensity of dehydrogenase and acid phosphatase activities as well as of the dynamics of selected groups of microorganisms in the soil under the cultivation of winter triticale following the application of the following seed dressings: (a.s.) flutriafol 2.5% + fludioxonil 2.5% in two doses and (a.s.) carboxin and tiuram.

MATERIAL AND METHODS

Investigations were conducted in 2007 on plots of Brody Agricultural Experimental Station of the Department of Soil and Plant Cultivation which belongs to Poznań University of Life Sciences.

The experiment was established using the random block design on plots of 28 m^2 area which were sown with cv. Viton of winter triticale (L.).

Soil samples employed to carry out biochemical and microbiological analyses were collected at five different dates (every month), which were connected with consecutive developmental phases of triticale: the 1st date – emergence (BBCH 01-09), the 2nd date – tillering (BBCH 20-29), the 3rd date – flowering (BBCH 61-69), the 4th date – dough phase (BBCH 83-89) and the 5th date – after harvest.

According to PTG classification [12], the soil from the experimental plots belongs to typical grey-brown podzolic soils developed from light loamy sands deposited shallowly on light clay. According to soil valuation classification, the soil was classified as IIIb–IVa class, whereas with respect to agricultural suitability, it was included in a very good rye complex. The soil from the experimental field was characterized by good phosphorus and potassium content and low content of magnesium with soil reaction close to neutral (pH = 6.2) (Tab. 1).

The following combinations were applied in the experiment: 1. control (plants without seed dressing), 2. seed dressing a.s. flutriafol 2.5% + fludioxonil 2.5% 150 ml/100 kg grain, 3. seed dressing a.s. flutriafol 2.5% + fludioxonil 2.5% 200 ml/100 kg grain, 4. vitavax 2000 FS (a.s., carboxin + tiuram 300 ml/100 kg grain). Before sowing,

Table 1. Characteristics of the basic chemical composition of the soil

Soil horizon (cm)	pH	% C_{org}	% humus	C:N	Magnesium mg $MgO \cdot 100g^{-1}$	Phosphorus mg $P_2O_5 \cdot 100g^{-1}$	Potassium mg $K_2O \cdot 100g^{-1}$
0-30	6.2	0.81	1.39	10.7	6.4	40.5	16.7

a multi-component fertiliser Agrofoska was applied 0–24–24 at the dose of 250 kg/ha, whereas post-sowing – 308 kg/ha ammonium saltpetre was used.

The climatic conditions in the year when investigations were carried out favoured good triticale productivity (Tab. 2).

Enzymatic activity

Examination of the soil enzymatic activity treated with various pesticides was based on the determination of the activity of:

- Dehydrogenases by spectrophotometric method using as substrate 1% TTC (triphenyltetrazole chloride) after 24-hour incubation at the temperature of 30°C and 485 nm wave length and was expressed in mmol TPF·kg^{-1}d.m. of soil·24 h^{-1} [5].
- The activity of acid phosphatase was detrmined using as substrate p-nitrophenylphosphate sodium, after one hour incubation at 37°C with wave length 400 nm. Enzyme activity was expressed in mmol PNP·kg^{-1}·h^{-1} [6].

Table 2. Meteorological conditions during the vegetation season of winter triticale

MONTHS	TEMPERATURE/ PRECIPITATION	DECADE			MEAN/ TOTAL
		I	II	III	
September	Mean temperature in °C	18.6	18.8	17.0	18.1
2007	Total precipitation in mm	61.7	4.2	5.0	70.9
October	Mean temperature in °C	13.1	12.8	13.6	13.2
2007	Total precipitation in mm	23.4	4.1	21.3	48.8
November	Mean temperature in °C	10.4	8.0	6.3	8.2
2007	Total precipitation in mm	3.5	17.1	0.7	21.3
December	Mean temperature in °C	13.1	12.8	13.6	13.2
2007	Total precipitation in mm	43.4	12.2	12.1	67.7
January	Mean temperature in °C	5.4	0.4	-1.9	1.3
2008	Total precipitation in mm	44.4	3.6	1.7	49.7
February	Mean temperature in °C	-1.2	3.7	4.5	2.3
2008	Total precipitation in mm	17.3	57.0	38.7	113
March	Mean temperature in °C	4.2	2.0	6.3	4.2
2008	Total precipitation in mm	6.4	1.5	22.6	30.5
April	Mean temperature in °C	5.2	4.3	3.0	4.2
2008	Total precipitation in mm	22.9	29.5	23.3	75.7
May	Mean temperature in °C	6.5	7.5	12.0	8.7
2008	Total precipitation in mm	49.2	71.5	0.0	120.7
June	Mean temperature in °C	14.0	15.1	16.4	15.2
2008	Total precipitation in mm	1.4	18.1	0.0	19.5
July	Mean temperature in °C	21.0	16.7	19.7	19.1
2008	Total precipitation in mm	0.0	4.2	4.4	8.6

Microbiological analyses

Using the appropriate agar medium (in five replications), numbers of microorganisms were determined in soil samples collected from the depth of 15−20 cm in inter-rows from under the plants with the assistance of the flooded plates dilution method according to Koch. Mean numbers of colonies were converted into soil dry matter:

- Total number of bacteria were determined on a ready-to-use Merck-Standard count agar following 5-day incubation at the temperature of 25°C,
- Fungi were determined on a Martin medium following 5-day incubation at the temperature of 24°C [11]
- Copiotrophs were determined on an NB (nutritive broth) medium following 5-day incubation at the temperature of 25°C [6] ,
- Oligotrophs were determined on a DNB (diluted nutritive broth) medium following 21-day incubation at the temperature of 25°C [6]
- Numbers of Azotobacter were determined placing on a Petri dish 1 g of the soil sample which was mixed with a medium according to Jensen [3]. Plates were incubated for 5 days at the temperature of 24°C.

Plants were harvested with the assistance of a Wintersteiger plot combine harvester from the area of 15 m^2 of the plot. The yield result was converted into a constant for cereals of 15% moisture content and was given in t/ha.

Statistical analysis

All the obtained results were subjected to formal evaluation in analyses of variations adequate to the experimental design. The results of field experiments were assessed in analyses for multiple experiments established in completely randomised block systems. The synthetic elaboration of the results of field experiments employed a complete procedure of testing of inter-object variability to the mean experimental error and to environmental interaction. All general F tests and specific t tests were carried out at the significance level of $\lambda = 0.05$.

RESULTS AND DISCUSSION

Analysis of the impact of seed dressings on enzymatic activity

Results of many performed experiments corroborated that enzymes, i.e. dehydrogenases (DHA) and acid phosphatase (PHOS-H), provide very good parameters for the evaluation of the impact of the applied pesticides on soil microbiological activity. Changes in soil enzymatic activity can constitute a reflection of the composition of the microbial population and functional variability and, therefore, can be used as indicators of environmental contamination [19].

Figure 1 presents the obtained results of the experiment examining the impact of the applied seed dressings on the activity of dehydrogenases (DHA) in soil. The analysis of these results indicates a change in the activity of the examined soil enzymes depending on the seed dressing applied in the experiment as well as at the date of investigations. However, the performed statistical analysis revealed that the recorded results were statistically significant only at the second date. The applied seed dressing (a.s., flutriafol 2.5% + fludioxonil 2.5%) in both doses exerted a stimulating effect on the dates 2nd and 3rd. On the other hand, on consecutive dates (the 4th and 5th),

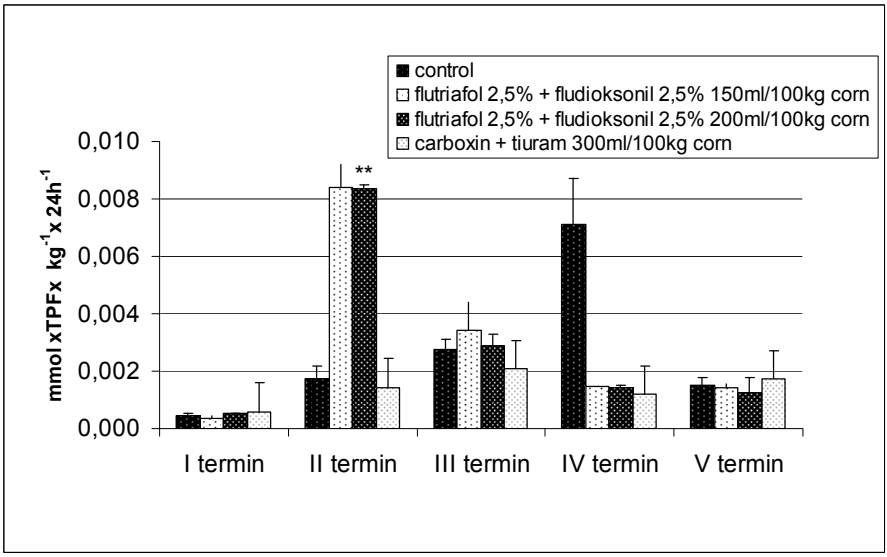

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Fig. 1. The effect of seed dressing on dehydrogenases activity

a decline in the activity of the examined dehydrogenases was observed as a result of the treatment of the discussed dressing in comparison with the control. The observed drop in the activity of soil enzymes on the last dates of investigations can be attributed to a more toxic influence of products of pesticide decomposition in relation to the original compound which entered the soil.

Vitavax 2000FS fungicide inhibited dehydrogenase activity at the 2nd, 3rd and 4th dates of investigation.

Results of investigations carried out by numerous researchers indicate a decline in the activity of dehydrogenases following the application of various pesticide preparations.

Studies on the impact of the Eminent 125 SL fungicide on dehydrogenase activity revealed a significant decline in the activity of these enzymes in sandy soils. On the other hand, the above compound used in clay soil resulted only in a short-term inhibition of the activity of dehydrogenases [4].

A decline in dehydrogenase activity following soil application of the Maneb fungicide was reported by Pozo *et al*. [17]. However, these researchers also reported increased activity of these enzymes following the application of Mancozeb fungicide.

Increased activity of dehydrogenases following soil application of the Maneb fungicide was reported in experiments carried out by Jastrzębska and Kucharski [8]. However, the above authors emphasised the fact that a dose 100 times higher than the recommended one inhibited the activity of those soil enzymes.

On the basis of the performed statistical analysis (Fig. 2−5), a positive correlation was found between the activity of dehydrogenases and total bacterial counts in the case of the second and third treatments when the flutriafol 2.5% + fludioxonil 2.5% seed dressing was applied in both doses (Fig. 3−4). The level of dehydrogenase activity in the course of the discussed experiment was probably associated with the development phase of plants

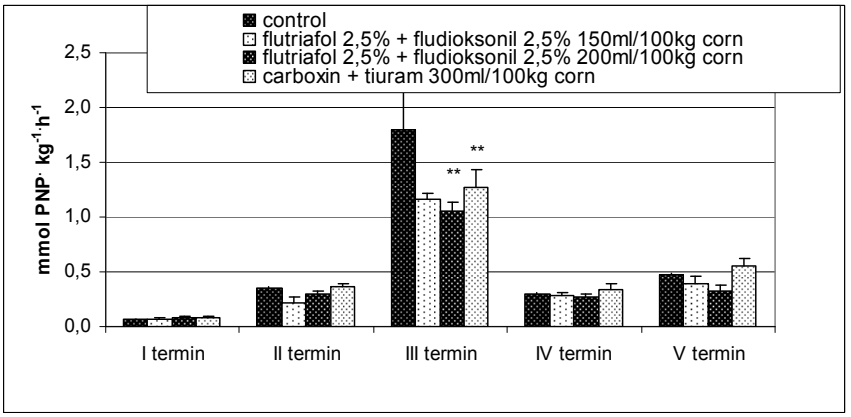

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Fig. 2. Relation between the total bacteria number and the dehydrogenases activity in control soil

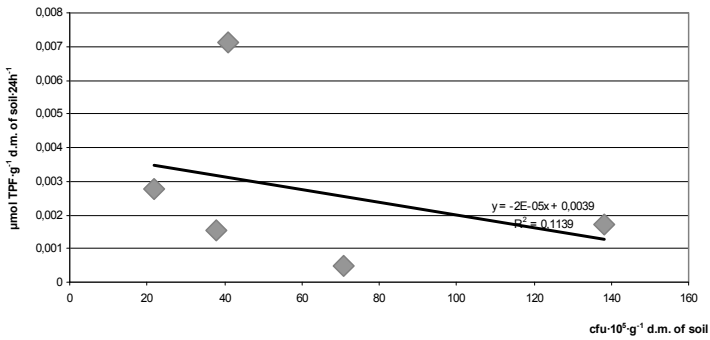

Fig. 3. Relation between the total bacteria number and the dehydrogenases activity in soil (flutriafol 2,5% + fludioksonil 2,5% 150 ml/100 kg corn)

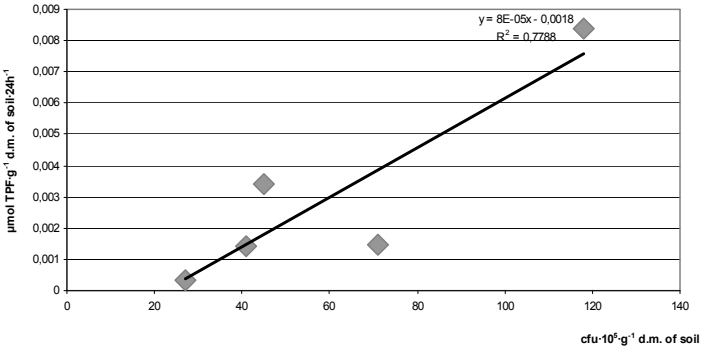

Fig. 4. Relation between the total bacteria number and the dehydrogenases activity in soil (flutriafol 2,5% + fludioksonil 2,5% 200 ml/100 kg corn)

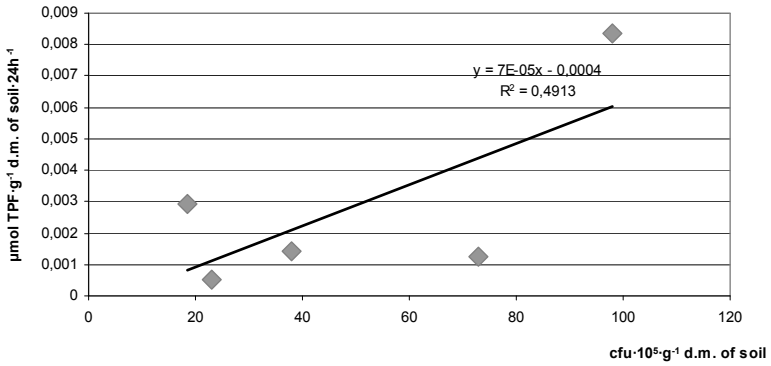

Fig. 5. Relation between the total bacteria number and the dehydrogenases activity in soil (karboksyna + tiuram 300 ml/100 kg corn)

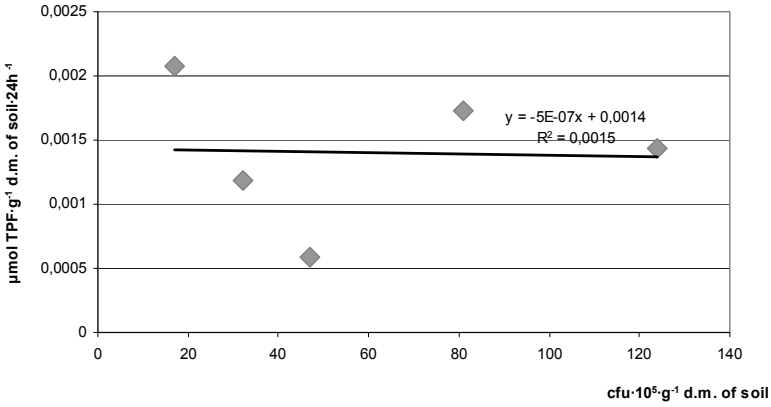

Fig. 6. The effect of seed dressing on the acid phosphatase activity

as well as with the presence of co-metabolites from the applied seed dressing which could have provided a good carbon source for bacteria. Differing levels of enzymatic activity associated with development phase of plants are recorded in the studies by Niewiadomska *at al* [15].

Figure 6 presents the obtained results of the experiment investigating the influence of the applied seed dressings on the activity of the acid phosphatase (PHOS-H) activity in soil. The performed investigations proved that the action of fungicides did not remain neutral with respect to the activity of the assessed soil enzyme and depended on the date of the performed analysis as well as on the kind of the applied preparation.

The performed statistical analysis revealed a significant impact of the applied preparations only at the 3rd date of analyses. The recorded decline in the activity of acid phosphatase in soil at the above date – in relation to the control – amounted to, respectively: 9.4% – following the application of the (a.s.) flutriafol 2.5% + fludioxonil 2.5% 150 ml/100 kg grain seed dressing, 41.6% – after the application of the (a.s.)

flutriafol 2.5% + fludioxonil 2.5% 200 ml/100 kg grain seed dressing and 29.4% – when Vitavax 2000 FS (a.s., carboxin + tiuram 300 ml/100 kg grain) seed dressing was applied.

A negative impact of plant protection preparations on the activity of acid phosphatase in soil was also confirmed by other literature data [22, 23, 24].

Acid phosphatase activity inhibition in soil following the application of three examined herbicides: Solar 200 EC, Lontrel 300 SL and Mustang 306 SE was also reported by Nowak *et al*. [16].

Analysis of the impact of seed dressings on numbers of selected groups of soil microorganisms under triticale cultivation

Numerous investigations indicate that pesticides introduced into the soil can cause quantitative and qualitative changes in soil microfloral composition [22, 2]. The introduced xenobiotics lead to distinct qualitative changes in the composition of soil microflora. Frequently, certain species of microorganisms are eliminated while, at the same time, other species of microorganisms take over [14].

The performed experiment demonstrated that the total bacterial counts in the soil underwent fluctuations depending on the developmental stage of triticale and the applied fungicide. The conducted statistical analysis showed that the employed preparations failed to exert a statistically significant influence on total bacterial counts in the soil. At the first date of analyses (emergence), the seed dressing (a.s. flutriafol 2.5% + fludioxonil 2.5%) applied at the dose of 150 ml/100 kg grain resulted in a 50% decline of the discussed group of bacteria in comparison with the control, while the higher dose of the same preparation decreased their number by 64%. On the other hand, the application of Vitavax reduced numbers of bacteria by 30% (Fig. 7).

At the consecutive date of analyses, a declining trend of total bacterial counts was also observed, although this tendency was less intense. At the 3rd and 4th dates of analyses, total numbers of bacteria following treatment with seed dressing (a.s. flutriafol

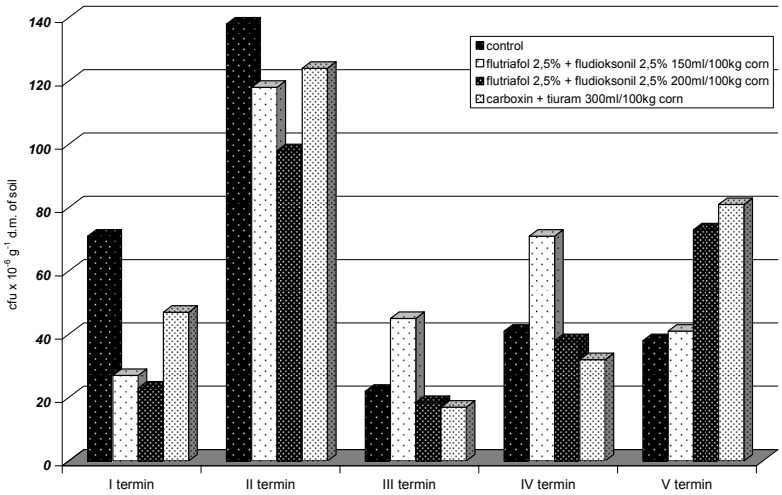

Fig. 7. The effect of seed dressings on the number of the total bacteria

2.5%) applied at the lower dose were found to have increased, whereas at the 5th date, all the applied seed dressings exerted a stimulating influence on total bacterial counts in comparison with the control.

When investigating the impact of Funaben T fungicide and Pivot 100 herbicide on total bacterial counts, Niewiadomska [14] also reported a decline in numbers of the discussed group of microorganisms at the first dates of analyses in comparison with the control, but later on their numbers increased again.

Similarly to total bacterial counts, also numbers of oligotrophic bacteria in soil underwent fluctuations depending on the applied seed dressing as well as on the date associated with the developmental stage of the examined winter triticale (Fig. 8).

At the first two dates of analyses, a declining trend was observed for all the applied seed dressings. On the other hand, from the 3rd date of analyses (flowering), the number of oligotrophs affected by the applied 150 ml/100 kg grain fungicide (a.s. flutriafol 2.5% + fludioxonil 2.5%) was by 80% higher in comparison with the control, whereas in the case of the same seed dressing applied at a higher dose – by 65% higher. Also, the application of Vitavax (a.s. carboxin + tiuram 300 ml/100 kg grain) caused stimulation of numbers of the discussed group of microorganisms from the same date, while at the 5th date, it affected their numbers statistically significantly.

In their experiments on the impact of Oxafun T fungicide on soil microorganisms, Kaszubiak and Durska [9] demonstrated a 54% increase of oligotrophs in the course of the trial. They proved a positive influence of the applied preparation which could have provided a source of carbon and nitrogen. On the other hand, Jastrzębska and Kucharski [7] drew different conclusions on the basis of their experiments in which they investigated the effect of Unix 75 WG and Swing Top 183 SC fungicides and recorded a decline in numbers of soil oligotrophs.

Depending on the date of the performed analyses as well as the applied seed dressing, also numbers of copiotrophs found in the soil were observed to have changed. The

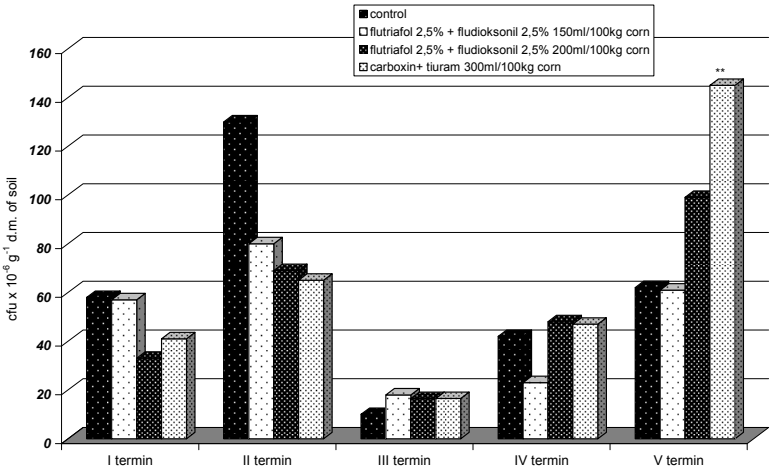

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Fig. 8. The effect of seed dressings on the number of oligotrophic bacteria

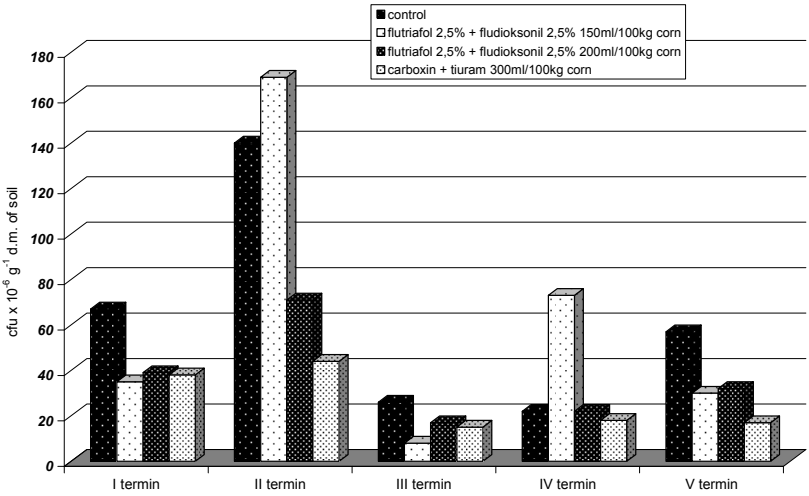

Fig. 9. The effect of seed dressings on the number of copiotrophic bacteria

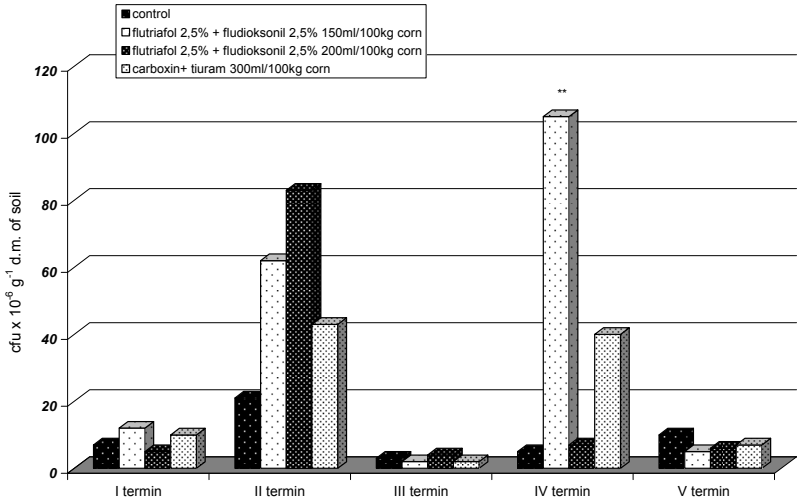

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Fig. 10. The effect of seed dressings on the number of fungi

employed preparations failed to impact these microorganisms statistically significantly, nevertheless majority of the obtained results indicate a negative influence of fungicides on the quantities of copiotrophic bacteria in soil (Fig. 9).

The only exception included the 2nd and 3rd dates of analyses when the fungicide (a.s. flutriafol 2.5% + fludioxonil 2.5%) was applied at the dose of 150 ml/100 kg grain and numbers of copiotrophic bacteria in comparison with the control increased by 20 and 60%, respectively.

In their investigations on the effect of fungicides on numbers of copiotrophs, Jastrzębska and Kucharski [7] reported a positive impact of fungicide preparations Swing Top 183 SC and Unix 75 WG (used in optimal doses). The first of these pesticides increased numbers of the discussed bacteria by 20% in a sown soil and by 17% in soil which was not sown. On the other hand, Unix 75 WG caused increase of copiotroph numbers by 48% in a sown soil and by 20% in an unsown one.

Numbers of fungi in the examined soil fluctuated depending on the employed seed dressing compound as well as the date of application connected with the developmental stage of the cultivated plant (Fig. 10). The performed analyses of results indicated a growing trend in fungi numbers.

The dose of 150 ml/100 kg grain of the seed dressing (a.s. flutriafol 2.5% + fludioxonil 2.5%) led to an almost twofold increase in numbers of the discussed group of microorganisms in comparison with the control. At the consecutive date of analyses, numbers of fungi were found to have increased in the case of all the applied seed dressings. Statistically significantly stimulating impact of fungicides on numbers of the discussed group of microorganisms was recorded at the 4th date of analyses. It was only at the 5th date of analyses that numbers of fungi halved in combinations with the applied fungicides in comparison with the control. The observed decline in numbers of fungi at this date could have been caused by the fact that, after the application of the fungicide, some species of fungi disappeared and were replaced by other species of microorganisms which was confirmed by the fact that at this date – apart from the drop in numbers of fungi – total bacterial and oligotroph counts in soil increased (Fig. 7, 8). Numerous investigations indicate that some fungicides lead to a change in the qualitative composition of soil fungal microflora. Following the application of fungicides, we can frequently observe loss of the part of fungal species and ecological succession of other species of microorganisms [18].

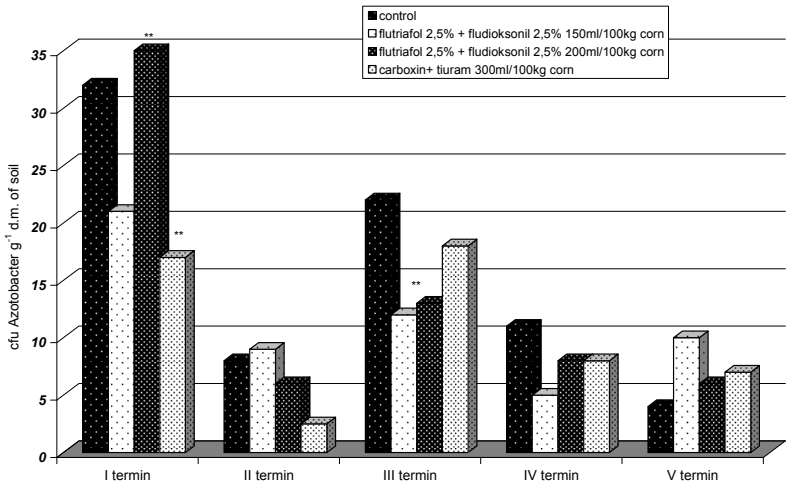

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Fig. 11. The effect of seed dressings on the number of Azotobacter

In addition, the employed pesticides also affected growth of bacteria from the *Azotobacter* genus. The lower dose of the applied seed dressing (a.s. flutriafol 2.5% + fludioxonil 2.5%; 150 ml/100 kg) as well as Vitavax (a.s. carboxin + tiuram 300 ml/100 kg) reduced highly significantly counts of *Azotobacter* bacteria at the 1st date of analysis Fig. 11). At the consecutive three dates, numbers of these microorganisms in soil decreased already at all the applied seed dressings. At the last date of analyses, the applied seed dressings exerted a stimulating influence on counts of *Azotobacter* bacteria.

In the literature many papers on the effect of fungicides on nitrogen fixing bacteria from the *Azotobacter* genus can be found. Some active ingredients of commonly applied preparations are toxic for *Azotobacter* cells, especially during initial stages, immediately after their application, although later on this toxicity decreases [13, 14]. The impact of Unix 75 WG and Swing Top 183 S.C. fungicides on *Azotobacter* development was investigated by Jastrzębska and Kucharski [7] who reported a decline in numbers of the discussed group of bacteria when 10 and 100 times higher doses were applied. On the other hand, Wyszkowska [22] demonstrated that among the examined groups of microorganisms, bacteria from the *Azotobacter* genus turned out to be most sensitive to pesticides employed in the experiment.

Frequently, the applied plant protection preparations clearly alter the qualitative composition of soil microflora. The elimination of certain groups of organisms results in a simultaneous ecological succession of other species of fungi or bacteria [18] and, consequently, numbers of the soil microflora undergo changes.

Another method of control of the impact of the employed pesticides was the level of triticale yields (Tab. 3).

The obtained results show that the applied seed dressings failed to exert a statistically significant influence on plant yields as well as on the mass of 1000 kernels. Nevertheless, it is evident that the above-mentioned parameters were always higher in combinations with the applied seed dressings.

Table 3. Mass of 1000 kernels and grain yields of winter triticale

No	Experimental objects	Dose/100 kg grain	Mass of 1000 kernels			Grain yield*		
			g	Increase in gelation to control		t/ha	Increase in gelation to control	
				g	%		t/ha	%
1	Control	–	36.08	–	**100**	13.6	–	**100**
2	Vitavax 200 FS	300 ml	37.50	1.42	**103.9**	14.0	0.4	**103.0**
4	*Flutriafol 2.5 % Fludioxonil 2.5 %*	150 ml	36.41	0.33	**100.9**	14.2	0.6	**104.2**
5	*Flutriafol 2.5 % Fludioxonil 2.5 %*	200 ml	37.00	0.92	**102.6**	13.9	0.3	**102.6**
LSD 0.05			Non-significant difference			Non-significant difference		

*grain yield converted to 15% moisture content in relation to all objects

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