

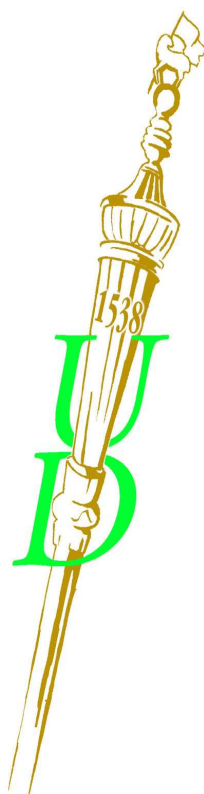
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PhD thesis

**EFFECT OF HERBICIDES USED IN MAIZE CULTURE
ON SOIL MICROBIOLOGICAL ACTIVITY**

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1. Introduction and goal set

The maize is one of the most developing dynamic cereal crops of the world, in the last fifteen years increased the maize plant production of the world by nearly 70%. Sowing area more than 157 million hectares, and more than 784 million tons grain crops are produced in the world as food, fodder and crops for industrial consumption.

Our country has one of the largest sowing areas in Europe, and also the country has a good place on the world ranking list in the maize production per capita. Among the arable land plants the maize has the largest sowing areas in Hungary, in 2008 it was nearly 1,2 million hectares. In the plant production indispensable the plant protection, it is important besides the protection against the pathogens and pests, in the regulation of weeds. Along the regulation of weeds, the pesticides applied are in connection with the soils. The pesticides effect on soil and organisms living in the soil. The pesticides applied in the surface effect at once, but those chemicals which are sprayed on plants also effect on soil, but its effect is longer and depends on the weather.

Herbicides play a very important role in the regulation system of weeds. More than 66% of the land of Hungary is used for agricultural production and about 19% is used for silviculture. The plant protection based on the chemicals is wide ranging in the agriculture. In Hungary 292 different pesticides could use in 1976-ban, in 1990 the number of chemicals used was near 900, and the 45% of pesticides was the herbicide. The number of permitted plant agent chemicals decreased to 765 by 2008, from this the herbicide is 41%, insecticide is 21% and the fungicide is 37%.

The herbicide usage is inseparable part of the plant production, but beside the exemption from weeds we have to count another secondary effect of chemicals on the soil life and on the so called “not purposed” soil organisms. In the Environmental World Conference of United Nations the pesticides was named among the most polluted agent of the world, they pollute intensively the atmosphere, the surface and the soil waters and the soils too. Nowadays new herbicides put into issue, their selectivity is better, than in case of the earlier chemicals, so they can be used in smaller concentration.

In the dissertation we would have like to know whether the herbicides applied in maize culture to the soil surface (Acenit A 880 EC, a Frontier 900 EC, Merlin 480 SC and a Wing EC), what kind of effect on the living microorganisms of soil and on the soil microbiological processes.

In laboratory circumstances the effects of herbicides were examined:

- on the number of total bacteria and on the growth of microscopical fungi (2004).

In small plot experiment the effects of herbicides were studied (2005-2008):

- on the number of total bacteria, and microscopical fungi,
- on the quantitative changes of aerobic cellulose decomposing bacteria and nitrifying bacteria,
- on the soil respiration, and the nitrate mobilization,
- on the quantity of fumigation-incubation biomass carbon, and fumigation-extraction biomass nitrogen, they were measured in different concentration of herbicides, in simple, in double and in fifth-dosages,

From the soil samples the quantity of herbicide-remains also was determined.

In small pot experiment (2008) the effect of Acenit A 880 EC and Merlin 480 SC were studied on the parameters mentioned above, and on the biomass quantity of a test –plant.

2. Material and Methods

2.1 In vitro investigation the herbicide-sensitivity of microorganisms

The laboratory investigation was done in the soil microbiological laboratory of Department of Agrochemistry and Soil Science. In the first period of the experiment, seven herbicides were chosen (Acenit A 880 EC, Frontier 900 EC, Gartoxin FW, Guardian Max, Merlin 480 SC, Trophy, Wing EC) these are used typically in maize culture. The first step was to investigate the effect of herbicide mixed to the culture media on the growth of some test organisms.

In first, to the herbicide containing agar-media 5 mm diameter micellium containing little disks were set with 3 different fungi species originating from stock-breeding (*Aspergillus niger*, *Fusarium oxysporum*, *Trichoderma sp.*) Four colonies “micellium disks” was set to every Petri-pot and they were incubated at 28 °C in thermostate. The diameter of fungi colonies was measured after 5 days, and the results were evaluated.

Secondly, to the herbicide containing Bouillon soup agar, soil suspension was pipetted originated from Calcareous chernozem (from the 10^6 dillution), they were incubated at 28 °C in thermostate and the total bacteria numbers were evaluated after two days.

At the bases of results of these experiment four herbicides were choosen from the seven for the following plough-land experiment.

2.2 Ivestigation the herbicide-sensitivity of microorganisms in small plot experiment in plough-land

The small plot experiment was set up at the experimental site of Debrecen University, Faculty of Agriculture, Department of Plant Protection on calcareous chernozem. The soil microbiological effects of herbicides were investigated under maize culture. The laboratory examinations were carried out in the soil chemical and soil microbiological laboratories of Department of Agrochemistry and Soil Science, from 2005-to 2008. The plots were set up in three repetitions. In the paper the results of three different dosages of four herbicides are shown regarding to homogenous average soil samples.

2.3 Ivestigation the herbicide-sensitivity of microorganisms in small pots

In order to prove the results of “impact-study”, in 2008 a small pot experiment was set up in the breeding house of the Department, where the soil moisture and the nutrient supply was secured in optimal level. In this experiment only those two herbicides was applied - Acenit A 880 EC and Merlin 480 SC – which previous effects had very large differences

2. 4. Presentation of herbicides used in the experiment

Table 1. Characteristic qualities of herbicides used in the experiment

Name of herbicides	Active ingredients	Content of active ingredient	Normal dose kg ha ⁻¹
Acenit A 880 EC	Acetochlor+AD 67 anthydotum	800 g * I ⁻¹ + 80 g * I ⁻¹	2,0 - 2,6
Frontier 900 EC	dimethenamide	900 g * I ⁻¹	1,2 - 1,6
Merlin 480 SC	isoxaflutole	480 g * I ⁻¹	0,16 – 0,2
Wing EC	dimethenamide+ pendimethalin	250 g * I ⁻¹ + 250 g * I ⁻¹	3,5 – 4,5

In the small plot experiment four herbicides were used, their characteristic qualities are the following: (Table 1.).

2.5 The important parameters of soil studied

The soil of experiment has loam texture. According to the $\text{pH}_{\text{H}_2\text{O}}$ (7,9) it is slightly alkaline. Among the chemical properties of soil, the CaCO_3 content was measured, according to the result its lime content is middle scale. The humus content and the soluble of phosphorus and potassium also were measured. The humus layer of soil is 70-80 cm; from it 40-50 cm contains humus uniformly. The under 30-35 cm layer goes to the parent material loess gradually. The humus content in the upper layer is 2,65%. According to nitrogen and phosphorus content the soil is middle supplied, in potassium the supplement of soil is in good category.

According to the international classification of soils (WRB), the soil of experiment is Calcic Endofluvic Chernozem (Endoskeletal).

2. 6. Soil microbiological parameters

Among the soil microbiological parameters the total numbers of bacteria, and the number of microscopical fungi was determined by plate dilution methods.

The number of aerobic cellulose decomposing bacteria and nitrifying bacteria was determined according to POCHON et al. (1962) with the MPN (Most Probable Number) method in liquid culture media. The basic condition of this MPN- method is the distribution of germs in the basic suspension should be equal. Because of the punctualities, five parallel inoculations have to be done.

The intensity of soil respiration (CO_2 -production of soil) can be measured by oxygen uptake of soil and the CO_2 -production. Along the measurement of soil respiration the CO_2 production was measured by absorption of CO_2 by NaOH- (HU et al 1997).

The quantity of microbial biomass-C was determined according to JENKINSON et al.(1976) by fumigation-incubation method. The microbial biomass-C was counted from the CO₂ –production of fumigated and not fumigated version of the same soil.

The biomass-N was measured by fumigation-extraction methods. Soil samples were fumigated by chloroform (kill the living microorganisms of soil), and the extraction was made by K₂SO₄. The N-content of filtrate was measured by Kjeldahl method, this result was convert into biomass nitrogen (BROOKES et al.1985).

The measurement of nitrate-mobilization the quantity of nitrate-nitrogen was measured from the fresh soil samples and after two week incubation of soil at 28°C. In the evaluation from the nitrate-nitrogen content of incubated soil subtract the nitrate-nitrogen measured from the fresh soil samples and the difference is the “nitrate-mobilization” (FELFÖLDY 1987).

2. 7. Applied statistical methods

The results were evaluated statistically by MS Excel program.

Regarding to the total number of bacteria, we counted with the number of colonies at the surface of Bouillon soup agar at 95%-authenticity interval. (HORVÁTH 1974).

To the evaluation of other results, two factors variance analysis was used to find whether the different qualitative and quantitative factors have significant effect on the soil parameters measured and calculating by the LSD5% values Sváb (1981).

The Pearson-type two-side correlation analyses also were performed (after the date were standardised), where relations were seeked among the different doses of herbicides and the soil parameters examined.

3. Evaluation of results

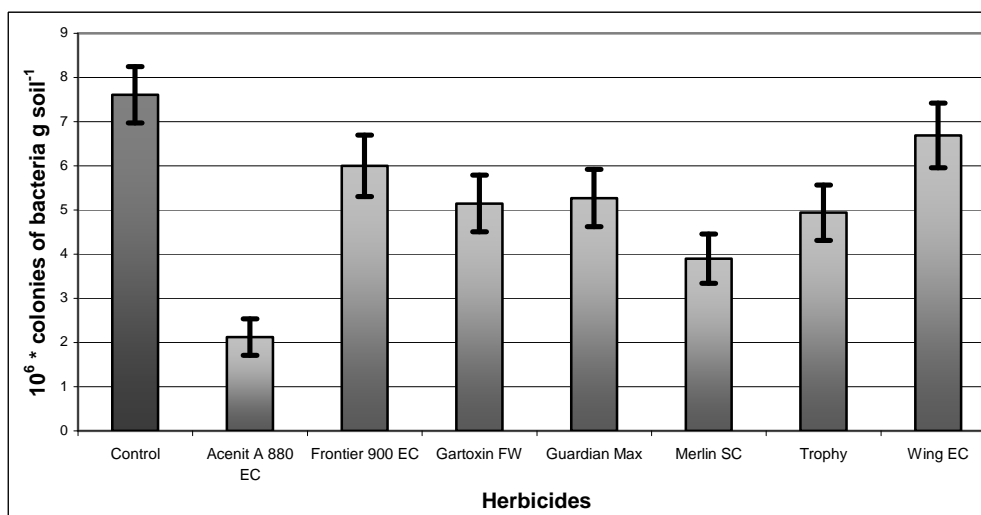
3.1 Investigation the herbicide-sensitivity in laboratory (2004)

In laboratory circumstances the effects of herbicides were examined:

on the number of total bacteria and on the growth of microscopical fungi (2004).

Evaluating the total bacteria number cultured in “poisoned Bouillon meat soup” the concentration effect couldn’t be separated so the results were evaluated uniformly at the bases of MPN (Most Probable Number) in 95% level. It can be explained that maybe the small concentration of herbicides have already killed the sensitive soil microorganisms, and do not kill the larger concentration of herbicide the resistant soil microorganisms. The number of bacteria at 95% reliability-level in the control was found $[6,97 - 8,25] \cdot 10^6$ colonies $\cdot g^{-1}$. In the culture media treated with Wing EC herbicide the number of bacteria approached the control level, so the herbicide effect was not significant regarding to this herbicide. By the effect of Acenit A 880 EC decreased the germ number drastically to the one third of control, $([1,71 - 2,54] \cdot 10^6 \text{ colony} \cdot g^{-1})$ while the germ number decreased by 50% in the case of Merlin SC (Figure 1.).

Figure 1. Effect of herbicides on the quantity of soil bacteria in calcareous chernozem soil (Debrecen, 2004)



In the experiment the growth of three microscopical fungi (*Trichoderma sp.*, *Aspergillus niger*, *Fusarium oxysporum*) was studied at different doses of herbicides compared to control. It was stated that the different herbicides inhibited the growth of fungi in different level. The diameters of fungi decreased significantly compared to the control, the results was proved statistically. It was interesting that only in the 40% of the treatments could be shown

significant differences between the fifth time dose and ten time dose of herbicides' effect on the fungi.

The growth of *Aspergillus niger* was inhibited by Acentit A 880 EC, and Frontier 900 EC herbicides, with the larger doses the inhibitor effect also increased. Regarding to the Gartoxin FW and Trophy, only little inhibiting effect was measured and even the larger doses of herbicides had no effect on the diameter of colonies.

The growth of *Fusarium oxysporum* was decreased by Acentit A 880 EC, and Frontier 900 EC, with the increased doses the inhibiting effect of herbicides also increased. The Wing EC and the Guardian Max was not influenced on the growth of fungi significantly, but there were differences in the colonies compare to control.

Regarding to *Trichoderma sp.* the extent of colonies was significantly inhibited by Frontier 900 EC and Acentit A 880 EC, with the increase of doses of herbicides, decreased the diameters of colonies significantly.

Totally it can be stated that in those culture media which contained Gartoxin FW, Guardian Max and Merlin SC, there was no significant decrease in the diameter of colonies compare to the control, while the treatments of Trophy and Wing EC even from the double doses decreased the diameters of fungi colonies. Except of the Frontier 900 EC and Acentit A 880 EC in the basic treatment of herbicides (1*) there was no significant decrease in diameter of colonies.

Summarizing and evaluating the results of two examinations mentioned above, four herbicides were chosen for further examination in plough-land circumstances. From the results it seems that Acentit A 880 EC and Frontier 900 EC had the largest inhibiting effects on soil microorganisms. The effect of Merlin 480 SC on soil microorganisms was not unambiguous, some times it had inhibiting effect, but sometimes it had neutral; while there was no inhibiting effect of Wing SC on soil microorganisms.

3.2 Effect of herbicides on the quantity occurrence and activity of soil microorganisms in a small plot experiment (2005-2008)

In the course of "impact-study" the number of total bacteria, the number of aerobic cellulose decomposing and nitrifying bacteria and quantity of microscopical fungi was determined. Besides the quantitative determination of soil bacteria and fungi, the effect of herbicides was

investigated on the soil microbiological activity (CO₂-production, quantity of microbial biomass-carbon and nitrogen, and to some degree of nitrate mobilization).

Finally the quantity of herbicide –remains also was determined from the soil samples.

Regarding to the total number of bacteria in the first three years (2005, 2006, and 2007) and in the first sampling time (Jun) the number of bacteria was smaller than in the second sampling time (July). In 2008 in every two sampling time the results were very similar regarding to the bacteria number. Second half of 2006 and the first six months of 2007 was very dry, there was very small precipitation in these periods, (135,6 mm and 171,5 mm), so relatively small bacteria number was measured. In Jun and July of 2008 there was considerable amount of precipitation (285 mm), in this period the number of soil bacteria was essentially larger.

Totally it can be stated that the number of total soil bacteria decreased by the effect of herbicides. The largest scale and in the most time inhibiting effect were measured in those soil samples which were treated by Acenit A 880 EC and Wing EC herbicides.

In 2005 the number of microscopical fungi was less by the effect of treatments of herbicides, than in the control parcel in every two sampling times. In the following three years (2006, 2007, 2008) – except some cases – by the effect of herbicide treatments increased the number of microscopic fungi. The concentration of herbicides also affected the number of fungi.

Regarding to the number of microscopic fungi, there was no negative effect of herbicides on the soil fungi, and what is more, in totally the number of fungi increased in the soil of herbicide treatments. It can be stated that number of microscopical fungi increased by the effect of herbicides, maybe the herbicides play as source of nutrient for fungi. In the largest scale the A 880 EC and Wing EC herbicides' effect was positive on the quantity of microscopical fungi.

In 2005 in every two sampling time, in 2006 and 2007 only in the first sampling time large quantity of aerobic cellulose decomposing bacteria was found in the treatments. In 2005, 2006 and 2008 in the first sampling time (Jun) the number of cellulose decomposing bacteria was higher in almost all treatments, compare to the control. In the second sampling time of these years and in 2007 (the driest year) there were hardly differences in the bacteria number between the control and the treatments. It seems that the larger doses of Wing EC and Acenit A 880 EC had a stimulating effect on the number of cellulose decomposing bacteria.

In 2005 in every two sampling time the number of nitrifying bacteria reached the ten thousand orders, this year was very favourable, but in the other years (2006, 2007, 2008) the number of nitrifying bacteria decreased to the thousand orders.

It is evident that in dryer years (2006, 2007) the number nitrifying bacteria smaller than in those years, when there is enough moisture in the soil, but it surprising, that their number was not higher significantly in 2008. There were eight soil sampling time, four herbicide and three different doses, it means ($8 \times 4 \times 3 = 96$) 96 effect-results. From this 96, in 28 cases there was inhibited effect, in 26 cases there was stimulating effect on the quantity of nitrifying bacteria. It is striking that in 2005 in the first, and 2006 in the second sampling time seven herbicides, and their different doses had stimulating effects, while in 2005 in the second, 2006 and 2008 in the first in the first sampling time in 7-9 cases had inhibiting effect on the number of nitrifying bacteria.

On the bases of results it can be stated that the Frontier 900 EC rather had inhibiting effect, while the Acenit A 880 EC had stimulating effects on the nitrification processes.

Regarding to soil respiration it seems that the herbicides and their different dosages had stimulating effect in 30%, and had inhibiting effect nearly in 15% on the CO₂-production. In 2006 Jun, (dry period) and 2008 Jun (wetter period) the CO₂-production was relatively small. In these series the results were similar to the control; only in some cases were a little differences in the CO₂-production. The most stimulating effect was measured in 2007, Jun, and the most negative effect was measured in 2005 Jun on the CO₂-production.

From the results it seems, that the Acenit A 880 EC and Merlin SC rather had stimulating, effect, while the Wing EC had inhibiting effects on the CO₂-production.

On the bases of results it may be concluded that among the soil microorganisms there were such type microorganisms, which could use the herbicides and their decomposition product as carbon sources, this is why the soil respiration increased in several treatments

In the course of “impact-study” regarding to the herbicides and their dosages it can be stated that the herbicides influenced about 70 % (34” positive effect and in 36% negative) the quantity of microbial biomass carbon. In 2005 and 2007 Jun hard inhibiting effect was experienced in the majority of treatments. Stimulating effect was measured in 2005 and 2008 in Jun from the soil samples. We couldn’t speak consistent and unambiguous effect of herbicides on the microbial-biomass carbon. The positive and negative effects are nearly the same regarding to the different dosages of herbicides and among the effect of herbicides too.

Regarding to soil microbial biomass-nitrogen it seems that the herbicides and their different dosages had influence 45%, (33 positive and 13 negative effects); the stimulating effect was higher, than the inhibiting effect. It is conspicuous that in 2005, 2006, and 2008 Jun, when the dominating effect is the stimulation. The stimulation effect of Ace nit A 880 EC and Wing EC is prominent. The effect of Frontier 900 EC rather was negative on the quantity of biomass-nitrogen. From the results it clears, that in 2007 and 2008 in the first soil sampling time were the smallest amount of microbial biomass-nitrogen in the soil samples.

Compare to the content of microbial biomass-carbon and microbial biomass-nitrogen of soils, it may be concluded that the herbicides caused bigger changes in the microbial biomass-nitrogen, than in the microbial biomass-carbon of soil.

In 2005, 2006 and 2008 Jun the results of nitrate mobilization showed high values, the differences among the treatments were clearer, but in case of other soil sampling time the results of nitrate mobilization stayed bellow the control. In some cases there were significant differences among the treatments, but in the doses of the certain herbicides there were no significant differences. In the majority of herbicides – independent of the doses applied – in 2005 in the first soil sampling and in 2008 in Jun and July considerable stimulating effect was measured, from the $3 \times 12 = 36$ cases in 31. In 2006 Jun the effect of herbicides was negative on the nitrogen mobilization. In the experiment in the course of “impact-study” regarding to the herbicides and their dosages it can be stated that the herbicides had stimulating effect in 48%, and inhibiting effect in 18% on the nitrate mobilization. The large doses of Frontier 900 EC herbicide and Acenit A 880 EC are pointed out because in these treatments increased the nitrate mobilization.

With reference to the results of small plot experiment set up with four herbicides, the following connection can be stated:

- The number of total soil bacteria decreased significantly by the effect of herbicides.
- The microbial biomass carbon, the aerobic cellulose decomposing bacteria as well as CO₂-production – with different strength depending on the type of herbicides – negative connection was experienced;
- Between the nitrate mobilization and aerobic cellulose decomposing bacteria, as well as the CO₂-production, positive correlation was proved.

It is shown in the table 2. that the herbicide-remains of acetochlor, dimethenamid and pendimethalin could be measured in the treatments and what is more from the control too. It may be concluded that the herbicides moving in the soil and this three month was not enough for the mineralization of certain herbicides.

Except of isoxaflutole every active ingredients could be measured even in the basic treatments, in the five time doses these results were larger by orders. The isoxaflutole ingredient could be shown only in the fifth time dose of Merlin 480 SC herbicide. This suggests that this active ingredient went through mineralization processes most quickly in the soil.

Table 2.Examination of herbicide-remains in the soils
(Debrecen, 2008)

Sample	Active ingredients mg*kg ⁻¹			
	acetochlor	dimethenamide	pendimethalin	isoxaflutole
Control	0,0002	0,0008	0,0006	-
Acenit A 880 EC 1*	0,0014	-	-	-
Acenit A 880 EC 5*	0,1244	-	-	-
Frontier 900 EC 1*	-	0,0202	-	-
Frontier 900 EC 5*	-	0,2013	-	-
Merlin 480 SC 1*	-	-	-	-
Merlin 480 SC 5*	-	-	-	0,0085
Wing EC 1*	-	0,0059	0,0010	-
Wing EC 5*	-	0,0117	0,1298	-
Instruments	GC-MS			LC-MS

To summarize the results of experiment, try to evaluate the cumulative effects of herbicides. In Table 9. the summing results can be shown, in the table only those results are build up, where the treatments caused significant positive or negative results.

The number of microscopic fungi increased in 55% of treatments significantly, and only in 21,9% decreased by the effect of herbicides according to the average results of four years.

The number of cellulose decomposing bacteria increased in most treatments, it can be stated that the increase was significant in nearly 50% of the treatments, in 40% there was no change, and only in 10% of treatments decreased the number of bacteria.

Regarding to nitrifying bacteria and soil respiration, similar results could be shown; the positive and negative effect was in 25-25% of treatments and there were no differences in

50% of treatments. Concerning microbial biomass carbon one-third and one-third was the positive, negative and neutral effect.

Table 3. Summing evaluation of herbicides' effect in the small plot experiment
(Debrecen, 2004-2008)

	Acenit A 880 EC			Frontier 900 EC			Merlin 480 Sc			Wing EC			Összesen		
Significant differences	+	-	0	+	-	0	+	-	0	+	-	0	+	-	0
Total number of bacteria	1	16	7	2	15	7	2	18	4	0	16	8	5	65	26
Number of microscopical fungi	14	5	5	13	4	7	13	6	5	11	6	7	50	21	25
Cellulose decomposing bacteria	11	3	10	9	4	11	12	3	9	14	3	7	46	13	37
Number of nitrifying bacteria	7	4	13	6	9	9	7	8	9	6	6	12	26	27	43
Soil respiration	8	5	11	6	3	15	8	3	13	6	6	12	28	17	51
Microbial biomass-carbon	9	7	8	10	10	4	8	12	4	7	7	10	34	36	26
Microbial biomass-nitrogen	10	3	11	4	7	13	6	4	14	11	1	12	31	15	50
Nitrate mobilization	12	3	9	14	8	2	12	3	9	9	4	11	47	18	31

The quantity of microbial biomass nitrogen –except in the treatments of Frontier 900 EC- the positive effects were more than the negative by the effect of herbicides. Totality in the 52% of treatments there were no significant differences among the results.

In case of nitrate mobilization, more positive effects were experienced than negative, except the Wing EC, where the effects was neutral. In totality, in the average value of four years the 50% of treatments of herbicides had stimulation effects on nitrate mobilization and only in 19% of treatment decreased the value of it.

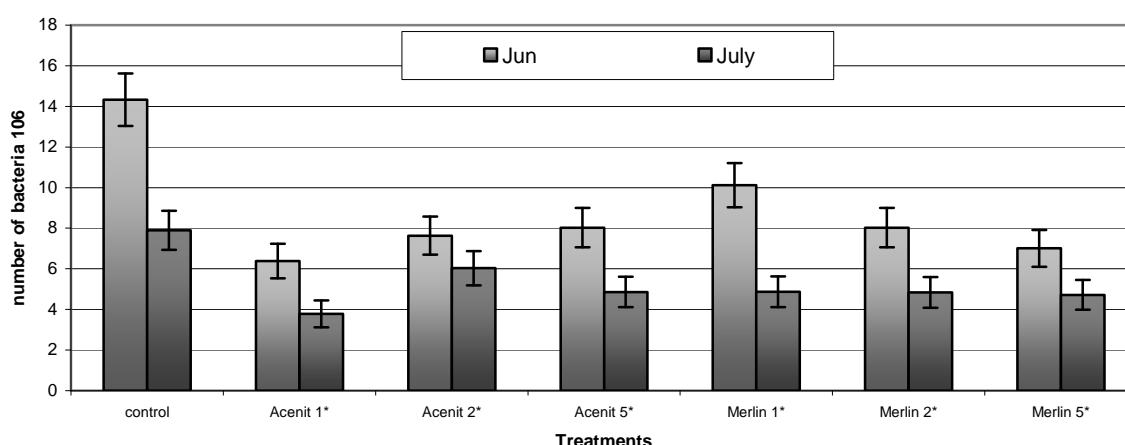
3.3. Effect of herbicides on the soil microbiological parameters in a small-pot experiment (2008)

It was very important for us to set up a small pot-experiment, because with the help of this method the microbiological processes taking place in the soil can be followed in exact

circumstances and the optimal nutrient and water supply for growth of maize plant can be provided.

In Jun the number of total bacteria was the half of the control in those pots, where the soil was treated by Acenit A 880 EC herbicide (Figure 2.), differences were significant. With the increase of doses, parallel increased the bacterium number, but this increase was not significant. Regarding to the effect of Merlin 480 SC herbicide, in these pots –similar to the previous results – the number of total bacteria was significantly smaller, than in the control. In July, in every treatment the bacterium number were significantly less compare to the control, except the double dose of Acenit. Regarding to the effect of Merlin 480 SC, every treatment of this herbicide resulted inhibiting effect significantly. It can be stated that the two herbicides and their all doses affected negatively to the number of total soil bacteria, the effect was significant.

Figure 2. Effect of herbicides on the number of total bacteria in the small-pot experiment
(Debrecen 2008 Jun and July)



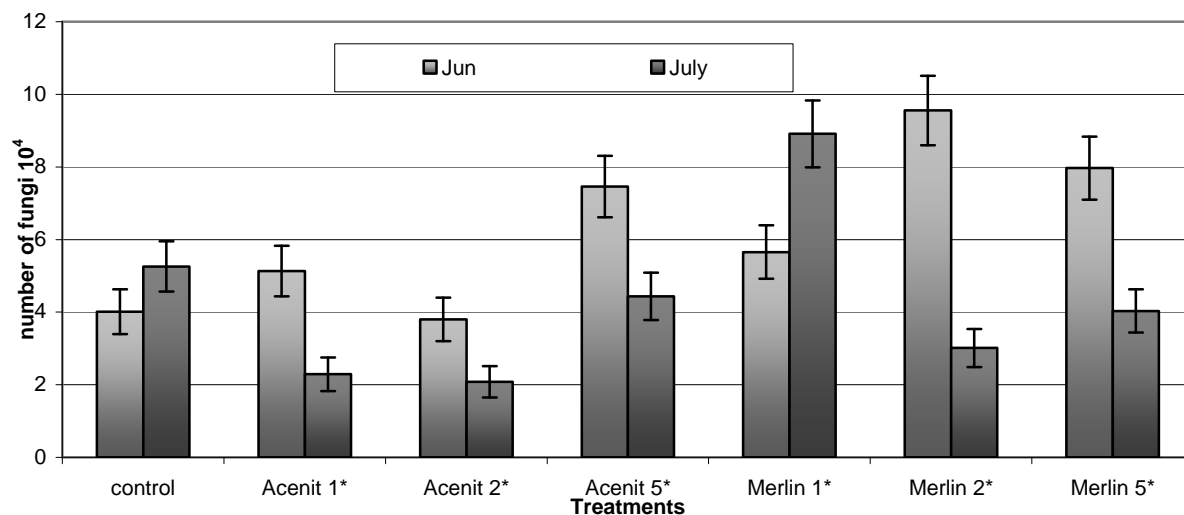
In Figure 3. the changes in the quantity of microscopical fungi can be shown in the small-pot experiment with the methods of MPN (Most Probable Number) in 95% level. In Jun the number of fungi was higher in all treatments, than in the control, except the double dose of Acenit. In July the highest number of fungi was measured in the control pots.

In the treatments containing Acenit A 880 EC the differences in the number of fungi were significantly smaller regarding to the two smaller doses. In the treatments containing Merlin 480 SC herbicide the number of microscopic fungi was also significantly smaller than in the basic treatment and in the control.

In the investigation of nitrate mobilization, the changes in nitrate-nitrogen content were measured after 14 days incubation of all two series of soil samples taken in Jun and July. In

the soil samples taken in Jun, the nitrate mobilization was higher in all treatments compare to the control. Regarding to Acenit 880 EC herbicide, the nitrate-nitrogen content increased with the increase of doses.

Figure 3. Effect of herbicides on the number of microscopical fungi in the small-pot experiment (Debrecen 2008 Jun and July)



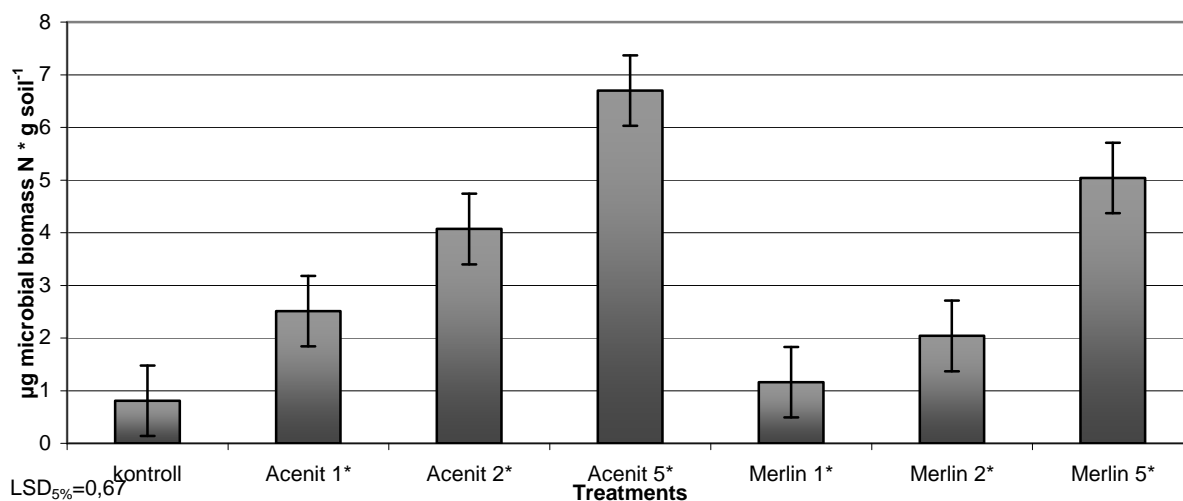
In the soil samples taken in July, among the treatments in the nitrate mobilization there were no significant differences only the basic treatment of Acenit A 880 EC herbicide had higher nitrate-nitrogen content compare to control. In the treatments containing Merlin 480 SC only the basic and five time doses decreased the nitrate mobilization significantly. Regarding to the nitrate mobilization, in six treatment stimulating effect, in seven treatments inhibiting effect could be experienced.

The CO₂-production, microbial biomass-carbon and microbial biomass-nitrogen were measured only from those soil samples, which were taken in second time. For the determination of this three soil microbiological parameters, an amount of soil is necessary, we could not take enough soils for investigation without the liquidation of the small-pots.

The respiration of soil was higher in all treatments compare to the control. It can be concluded from the results, that the basic treatments of two herbicide examined (Acenit A 880 EC and Merlin 480 SC) the soil respiration was significantly higher compare to control, in the other treatments there were no significant differences in the soil respiration.

The quantity of microbial biomass-carbon decreased significantly in the different treatments compare to the control, except the two middle-scale doses.

Figure 4. Effect of herbicides on the quantity of biomass-nitrogen in the small-pot experiment
(Debrecen 2008 Jun and July)



Regarding to the quantity of microbial biomass-nitrogen, the control pots had the smallest biomass-nitrogen, the results are very similar to the results in the plough-land experiment (Figure 4). In the treatments containing Acenit A 880 EC herbicide, with the increasing doses also increased the quantity of microbial biomass significantly compare to the control and also significant differences were measured among the results of different doses. In case of Merlin 480 SC with the increasing doses of herbicide increased the quantity of microbial biomass-nitrogen, but only the largest dose increased significantly this parameter.

Table 4. Effect of herbicides on the quantity of plant biomass in the small-pot experiment
(Debrecen 2008 Jun and July)

Treatments	Biomass of plants (g * plant ⁻¹)
Control	1,51
Acenit A 880 EC 1*	0,92
Acenit A 880 EC 2*	0,83
Acenit A 880 EC 5*	1,16
Merlin 480 SC 1*	1,37
Merlin 480 SC 2*	1,26
Merlin 480 SC 5*	0,67

The treatments of herbicides also affected the quantity of plant biomass (Table 4). It can be seen, that by the effect of Acenit A 880 EC decreased the plant-biomass in the pots in average by 30-50%. In the pots containing the basic dose of Merlin 480 SC there was no significant decrease in plant biomass, but in case of five times dose the plant biomass decreased by 60%.

4. New and novel scientific results

The environmental factors determine or influence the “impact-study”, so it is very important to approach the effects of herbicides with multi-side investigations. In laboratory conditions the influencing factors were decreased to minimum. In the small-pot experiment the conditions were kept under control (regular water and nutrient supply). In small plot experiment in plough land – besides herbicides - the climatic conditions also took part in the intensity of soil microbiological processes.

The herbicides decreased the quantity of bacterium population in chernozem soil generally, at the same time increased the number of microscopic fungi colonies.

From our results it seems that the quantity of microbial biomass nitrogen and the CO₂-production stayed at the control's level, so the treatments of herbicides were effectless to these biological parameters.

The nitrate mobilization increased in the most treatments of herbicides.

From the results it may be concluded that the Acenit A 880 EC and Frontier 900 EC influenced the values of soil microbiological activity in more frequently.

5. Results for practical utilization

- To get knowledge about the secondly effect of herbicides, it is important to make “impact-study”, and also expedient to determine the effects of herbicides on the population of microbes and biological activity of soil.
- The investigations proved that herbicide-remains can be found in the soil. The further accumulation of herbicides can affect on the soil biological processes as regards environment.
- At the bases of results it can be stated that the Merlin 480 SC herbicide has effect on soil microbial community rarely than other herbicides and smaller herbicide remains could be measured from soil. So we could recommend the practical application of Merlin 480 SC herbicide.
- Because of the accumulation of herbicide-remains and alternating effects of herbicide in the soil, we call the attention to the importance of soil monitoring examination system.

Publications in the field

Scientific periodicals:

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