Examination the effects of different herbicides on the soil microorganisms of a calcareous chernozem

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SUMMARY

Pesticides play a key role in fighting weeds, pests and parasitic fungi. According to surveys, pests reduce the yield of agricultural crops by 35% worldwide. Pests, fungi and weeds account for 14%, 12% and 9% yield loss, respectively (Gáborjányi et al., 1995). Chemicals have contributed to increasing and maintaining the yields of crop production for decades. Today, agricultural production (in spite of many efforts) is unthinkable without the use of pesticides (herbicides, insecticides and fungicides). On the other hand, these chemicals contribute to the pollution of the atmosphere, surface and underground waters, and agricultural soils, especially if they are applied improperly.

The sustainable agricultural production pays attention to environment-friendly cultivation-technologies; but at the same time it makes an effort to produce good quality and economical products. The examination of the herbicides' secondary effects, fits into this chain of idas namely, how the herbicides affect – stimulating or inhibiting – the soil microbiological processes, prevention of soil fertility.

In the course of the experimental work the effect of herbicides on soil biological properties were examined in different maize (Zea mays) cultures. We wanted wished to know that how the herbicides affect the quantity change of soil microorganisms, the life of different physiological groups of bacteria and the activity of microorganisms. A small pot experiment was set up in 2008 with the application of two herbicides - Acenit A 880 EC and Merlin 480 SC – in the breeding house of the Department. The moisture content and nutrient supply were at optimal level in the experiment.

On the basis of results the following can be stated:

1. *It can be stated that the two herbicides and all their doses affected negatively the number of total soil bacteria, the inhibiting effects were significant. The quantity of microscopical fungi increased by the effect of Merlin 480 SC and decreased in the treatments of Acenit A 880 EC.*
2. *The Acenit A 880 EC had stimulating effect on the nitrate mobilization. The CO_2-production was stimulated by the basic doses of herbicides; the other treatments did not influence the CO_2-production significantly.*
3. *The quantity of microbial biomass-carbon –except for only one treatment- decreased significantly by the effect of herbicides. Besides it, the quantity of microbial biomass-nitrogen increased significantly in the treatments of Acenit A 880 EC.*
4. *The biomass of test plant decreased in the treatments of herbicides, their quantities were smaller than in the control. In the pots treated by Merlin 480 SC, parallel with the increase of doses decreased the quantity of plant-biomass.*

INTRODUCTION

The maize is one of the most developing dynamic cereal crops of the world, in the last fifteen years the maize plant production of the world has increased by nearly 70% (Boros et al. 2008). Our country has one of the largest sowing areas in Europe, and also 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 (Nagy, 2009). In the plant production the plant protection is indispensable, 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 (Kádár, 2001). The pesticides effect on soil and organisms living in the soil. The pesticides applied on 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 (Lengyel, 2002).

The plant protection based on the chemicals is wide ranging in the agriculture. In Hungary 292 different pesticides could be used in 1976, in 1990 the number of chemicals used was near 900, and 45% of pesticides were herbicides. The number of permitted plant agent chemicals decreased to 765 by 2009, from these the share of herbicides was 41%, that of insecticides was 21% and that of fungicides was 37%.

The herbicide usage is inseparable part of the plant production, but apart from weed control we have to count another, secondary effect of chemicals on the soil life and on the so called "not purpose" soil organisms (Kecskés, 1976). When herbicides enter the soil, the sensitive organisms die and their easily decomposable residues are utilized by the survivors (Cervelli et al., 1978). Some organisms can directly utilize herbicides for their growth. In addition, the amounts of the organisms, which consume the metabolites of herbicide decomposing organisms and the decomposed chemical residues also increases. From the aspect of soil biology, neither inducing, nor inhibiting should be used permanently, since both groups have an effect on the microbial community and change the existing biological balance. Herbicides should be used which have only a minimal secondary effect on soil microbes should be used , in addition to their weed killing effect. The changes in the number and ratio of microorganisms are due to the transformation of species' biodiversity. The number of the more sensitive species reduces, certain species may even disappear, while species resistant to a certain herbicide can proliferate (Kapur et. al., 1981).

We should also count on the numerous side effects of the applied chemicals, which result in a reduction of soil fertility and yields (Vester, 1982). According to Müller (1991), herbicides can be classified into four groups based on their impacts on soil life: 1. inducing; 2. neutral (with no or hardly noticeable effect); 3. inhibiting; 4. not clarified effect.

Nowadays new herbicides are put into issue, their selectivity is better than that of the earlier chemicals, so they can be used in smaller concentrations (Inui et al., 2001).

Biró et al. (2005) studied the microbes of maize rhizosphere, which have a significant role in the nutrient uptake of maize; therefore, the studies of the effect of herbicides on the amount and activity of soil microbes are of great importance. The determination of the soil microbial biomass is aimed at assessing the microbiological status of the soil. It is a frequently-used method which is excellent for detecting changes (Szili-Kovács et al. 2005, 2006).

Acetochlor is an herbicide extensively used for weed control in several crops. It was first applied in 1994 in the USA and in 2000 in Europe. In the last years, acetochlor and its metabolites have appeared in surface and underground waters and in soils. Acetochlor and its metabolites are severe pollutants. It has become necessary to work out effective methods for the treatments and removal of the contaminations. (Sha-Yang Liu et al. 2004).

Oldal et al. (2005) studied different herbicide residues in soil and groundwater samples from special reference points of Hungary during winter. Atrazine was found in two of the 24 soil samples. However, in the ground water samples, acetochlor was also found among other materials in addition to atrazine.

The results of experiments carried out on calcareous chernozem soil suggest that during the vegetation period the herbicide Acenit caused significant changes in the numbers and enzyme activities of soil-living microbes (Kátai, 1998).

Kátai et al. (2003) found that herbicides containing acetochloric-atrasin generally increased the number of bacteria and microscopic fungi and enhanced CO_2 production. Experimental results about the other herbicides used in our investigation cannot be found in the literature.

Taylor-Lovell et al. (2002) revealed that the degradation of isoxoflutole in the soil is accelerated by microbes. Angerer et al. (2004) studied the effects of new type herbicides on microbes in model experiments in which herbicides were used in the some doses and higher ones them in agricultural practice. The results of these experiments show that microorganisms display different sensitivities to different doses of herbicides.

In small-pot experiments the effects of herbicides were studied:

- 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.

Samples were analysed in the laboratory of the UD CAS FA Department of Agrochemistry and Soil Science.

MATERIALS AND METHODS

In 2008 a small pot experiment was set up in the breeding house of the Department, where the soil moisture and the nutrient supply were secured at optimal level. In the small pot experiment two herbicides were used, their characteristic qualities are the following: (*Table 1.*).

Table 1.

Characteristic qualities of herbicides used in the experiment and the utilizing are doses

Name of herbicides	Active ingredients	Content of active ingredient	Normal dose kg ha^{-1}	Treatments		
				Simple dose cm^3 * small pot^{-1}	Double dose cm^3 * small pot^{-1}	Quintuple dose cm^3 * small pot^{-1}
Acenit A 880 EC	Acetochlor+AD 67 anthydotum	800 g * l^{-1}+ 80 g * l^{-1}	2,0 - 2,6	0,00353	0,00706	0,01765
Merlin 480 SC	isoxaflutole	480 g * l^{-1}	0,16 – 0,2	0,00027	0,00054	0,00135

The soil of experiment has loam texture. As for the pH_{H2O} (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 were also measured. The humus layer of soil is 70-80 cm from which 40-50cm layer contains humus uniformly, under 30-35cm layer graduates into the parent material to loess. The humus content in the upper layer is 2.65%. It's for the nitrogen and phosphorus contents the soil is middle supplied, regarding potassium supply the soil is in good category.

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

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

The number of aerobic cellulose decomposing bacteria and nitrifying bacteria were 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 that distribution of germs in the basic suspensions should be even. For the sake of exactitude, five parallel inoculations had to be done.

The intensity of soil respiration (CO_2-production of soil) can be measured by the oxygen uptake of soil and the CO_2-production. During 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. (1988) by fumigation-incubation method. The microbial biomass-C was counted from the CO_2 –production of the fumigated and not fumigated versions of the same soil.

The biomass-N was measured by fumigation-extraction methods. The soil samples were fumigated by chloroform (kill the living microorganisms of soil), and the extraction was made by K_2SO_4. The N-content of filtrate was measured by Kjeldahl method, this result was convert into biomass nitrogen (Brookes et al.1985).

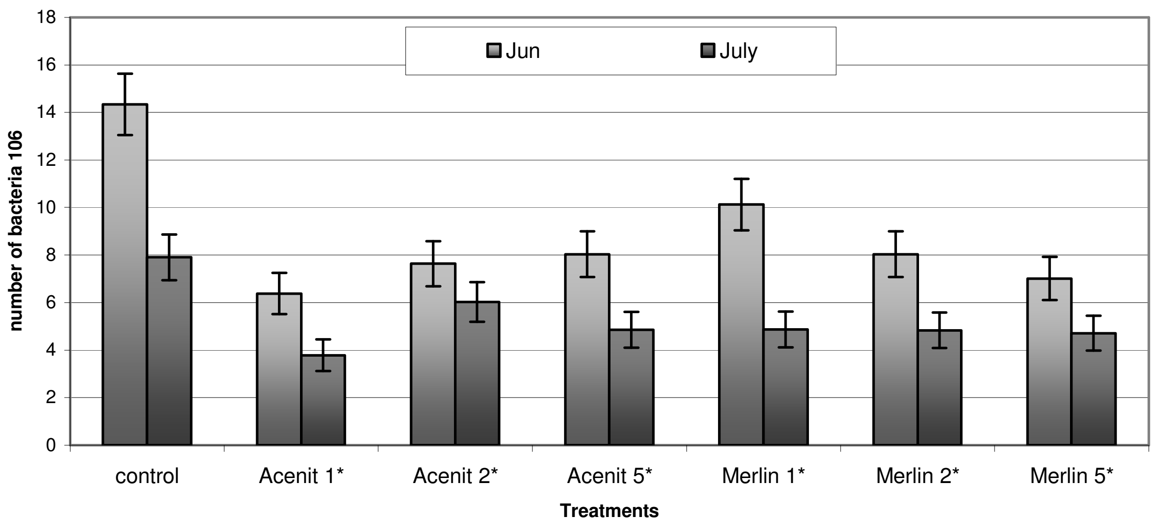
For the measurement of nitrate-mobilization, the quantity of nitrate-nitrogen was measured from the fresh soil samples and after two weeks incubation of soil at 28°C. In the evaluation from the nitrate-nitrogen content of incubated soil subtract the nitrate-nitrogen measured from the fresh soil samples and the difference is the "nitrate-mobilization" (Felföldy 1987).

RESULTS AND DISCUSSION

In Jun the number of total bacteria was the half of the control in the pots, where the soil was treated by Acenit A 880 EC herbicide (*Figure 1.*), and differences were significant. With the increase of doses, the bacterium number increased, but this increase was not significant.

Figure 1.: **Effect of herbicides on the number of total bacteria in the small-pot experiment**

(Debrecen 2008 Jun and July)



Regarding the effect of Merlin 480 SC herbicide, in these pots –similarly to the previous results – the numbers of total bacteria were significantly lower, than in the control. In July, in every treatment the bacterium number were significantly less compared to the control, except for the double dose of Acenit. Regarding the effect of Merlin 480 SC, every treatment of this herbicide resulted in inhibiting effect significantly. It can be stated that the two herbicides and all their doses affected negatively at the number of total soil bacteria, and the effect was significant.

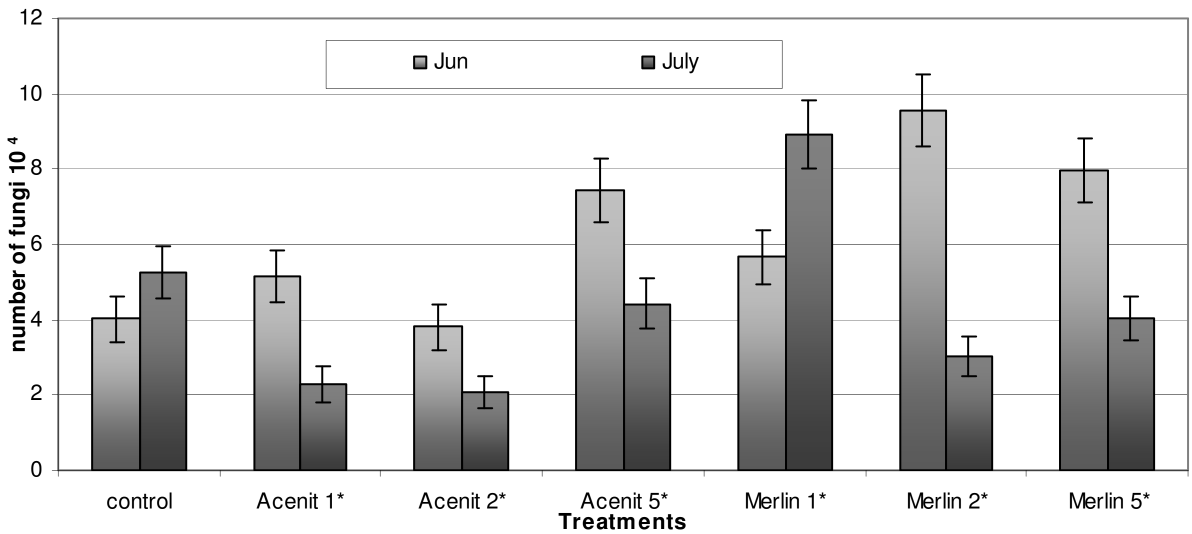
In *Figure 2*. the changes in the quantity of microscopical fungi can be seen in the small-pot experiment with the methods of MPN (Most Probable Number) at 95% level. In Jun the number of fungi was higher in all treatments than in the control, except for the double dose of Acenit. In July the highest 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 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.

For 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 June and July. In the soil samples taken in June, the nitrate mobilization was higher in all treatments compared to the control. Regarding Acenit 880 EC herbicide, the nitrate-nitrogen content increased with the increase of doses.

Figure 2.: **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, there were no significant differences among the treatments in the nitrate mobilization. Only the basic treatment of Acenit A 880 EC herbicide 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 nitrate mobilization, stimulating effects could be experienced in six treatments, while inhibiting ones in seven treatments.

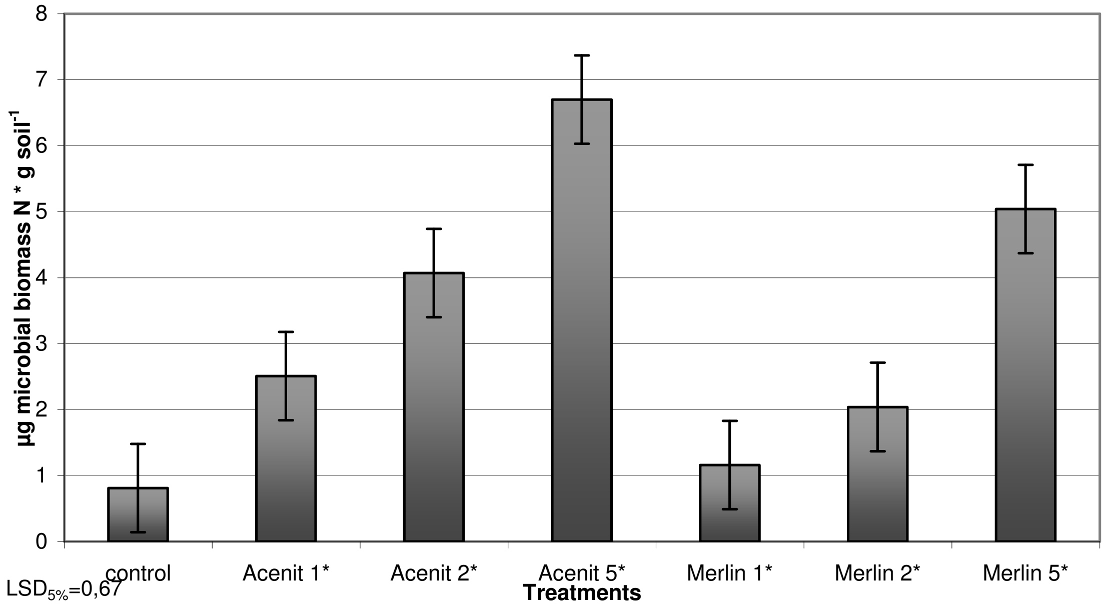
The CO_2-production, microbial biomass-carbon and microbial biomass-nitrogen were measured only from the soil samples which were taken for the second time. For the determination of this three microbiological parameters, an amount of soil is necessary, we could not take enough soil for investigation without the liquidation of the small-pots.

The respiration of soil was higher in all treatments compared to the control. It can be concluded from the results that in the basic treatments of two herbicides examined (Acenit A 880 EC and Merlin 480 SC) the soil respiration was significantly higher compared to control, while 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 compared to the control, except for the two middle-scale doses.

With reference to the quantity of microbial biomass-nitrogen, the control pots had the smallest biomass-nitrogen; the results are very similar to the results of the plough-land experiment (*Figure 3*). In the treatments containing Acenit A 880 EC herbicide, with the increasing doses the quantity of microbial biomass also increased significantly compared to the control and significant differences were also measured between the results of different doses. In the case of Merlin 480 SC, with the increasing doses of herbicide the quantity of microbial biomass-nitrogen increased, but it was only the largest dose that increased this parameter significantly.

Figure 3.: **Effect of herbicides on the quantity of biomass-nitrogen in the small-pot experiment**
(Debrecen 2008 Jun and July)



The treatments of herbicides also affected the quantity of plant biomass (*Table 2*). It can be seen that by the effect of Acenit A 880 EC the plant-biomass in the pots decreased by 30-50% on the average. In the pots containing the basic dose of Merlin 480 SC there was no significant decrease in plant biomass, but in the case of five times dose the plant biomass decreased by 60%.

Table 2.:

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

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