EFFECT OF "ACENIT (A 880 EC)" ON THE GROWTH OF MICROSCOPIC FUNGI AND MICROBIAL PROCESSES IN THE SOIL

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Abstract

We studied the effect of the herbicide "Acenit A 880 EC" with acetochlor as active ingredient, extensively used in corn production, on the growth of some microscopic fungi and on soil microbial processes under in vitro conditions.

The method of MILLER (1973) was applied, according to which Acenit was given to peptone-glucose-agar medium in different dosages (1x, 2x, 5x and 10x) and we studied the growth of the microscopic fungi: Trichoderma sp., Fusarium oxysporum and Aspergillus niger on the medium. In addition, we also determined how the bacterium population of calcareous chernozem soil changed under in vitro conditions as a result of the herbicide treatment. We studied the effect of different dosages of the herbicide on the nitrate exploration and CO_2 formation in calcareous chernozem soil after three and four weeks of incubation. The basic treatments were as follows: control, 1x, 2x, 5x and 10x Acenit dosages. Two series of experiments were set up: a) without glucose, b) treated with glucose.

Our results can be summarized as follows:

- *Even the regular (1x) dosage per hectare greatly inhibited the growth of Trichoderma sp. and Fusarium oxysporum colonies. The diameter of the colonies was halved when applying the tenfold dosage. Acenit did not inhibit so tremendously the growth of Aspergillus niger, but significant differences could be observed among the effects of the different dosages.*
- *As a result of the "Acenit A 880 EC" treatment, the bacterium population of calcareous chernozem soil reduced independently from the increasing concentration of the herbicide to about one third of that of the control (from [6.97-8.25]$\cdot 10^6$ colony* g^{-1} to [1.90-2.77]$\cdot 10^6$ colony* g^{-1}).*
- *When studying soil respiration, it was observed that CO_2 concentration reduced to two-thirds of the former concentration in a week as a result of the herbicide treatment regardless of the dosage. In the second and third weeks, no difference was detected between the control and the treated samples.*
- *Although the herbicide treatments did not result in significant differences in the samples treated with glucose, but the values increased in the first two weeks as compared to the control and reduced in the third week.*

During the incubation time, carbon-dioxide formation increased in both series. In the series treated with glucose a considerably higher amount of carbon-dioxide was released.

- *In the samples treated with Acenit, nitrate release was higher than in the control for all the four weeks and the inducing effect of the tenfold dosage was always outstanding.*

In the glucose-treated samples, the smaller and larger concentrations were of inducing and inhibiting effect, respectively, after the first week. Nitrate release was considerably smaller in the glucose-treated samples. A partial explanation of it can be that a considerable amount of the nitrate formed was used by the microbes to decompose the organic material (glucose).

Key words: acenit, fungi, microbial process, soil

INTRODUCTION

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.

In 1976, 292 different pesticides could be applied in Hungary. In the 1994, the number of available pesticides was 618, 45% of which were herbicides. The number of licensed pesticides reduced to 638 by 2006, herbicides, insecticides and fungicides represented 42%, 21% and 37% of this number, respectively (http:/www.NEOLAND. hu).

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.

Oldal et al. (2005) have studied different herbicide residues during winter in soil and groundwater samples from special reference points of Hungary. 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.

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. Such herbicides should be used which have only a minimal secondary effect on soil microbes, 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).

When the 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 amount of those organisms also increases, which consume the metabolites of herbicide decomposing organisms and the decomposed chemical residues. Taylor-Lovell et al. (2002) found that soil microorganisms accelerated the decomposition of izoxaflutol.

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 impact on soil life: 1. inducing; 2. neutral (with no or hardly noticeable effect); 3. inhibiting; 4. not clarified effect.

Angerer et al. (2004) studied in model experiments the effect of regular and higher than regular doses of new generation herbicides on the amount of some important soil microbe groups that can be grown on selective media. During incubation, it was proved that the microbe groups have a different sensitivity to specific herbicide dosages. According to Bíró et al. (2002), the nitrogen fixing bacteria proved to be the most sensitive groups of microbes.

Acetochlor is a 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 appeared in surface and underground waters and in soils. Acetochlor and its metabolites are severe pollutants. It has become necessary to work out such effective methods by which the pollutants can be treated and removed (Sha-Yang Liu et al. 2004).

Acenit A 880 EC is a herbicide with acetochlor as active ingredient. Its active ingredient content is 800 g/l. Its dosage in practice is 2.0-2.6 l/ha. It is antidoted with 80 g/l AD-67. It is of preemergent application. It kills mono- and dicotyledonous weeds, it is effective against annual monocotyledonous weeds. It is used primarily in corn, soy and potato cultures.

In experiments on calcareous chernozem soil, Acenit caused a significant change I the amount and enzymatic activity of soil microorganisms. Its regular and multiple dosages increased the activity of phosphatase, saccharase and catalase enzymes. In all dosages, Acenit inhibited the activity of urease as compared to the control (Kátai, 1998).

Accoding to the studies of Kátai et al. (2003), the herbicide combination with acetochlor and atrazine content (Erunit A 530 FW) generally increased the number of bacteria and microscopic fungi, the enzymatic activity and CO_2 production.

In our study, we report on the in vitro studies performed with Acenit A 880 EC of acetochlor content extensively used in corn. We studied the effect of Acenit on the growth of some microscopic soil-borne fungi and the amount of bacteria in calcareous chernozem soil.

We determined nitrate exploration and CO_2 formation in calcareous chernozem soil after three and four weeks of incubation. The basic treatments were as follows: control, 1x, 2x, 5x and 10x Acenit dosages. Two series of experiments were set up: a) without glucose, b) treated with glucose.

MATERIALS AND METHODS

The laboratory examinations were performed at the soil biology lab of the Department of Agrochemistry and Soil Science of the UD CAS FA. For studying the growth of microscopic fungi, the method of Miller (1972) was used, which method (toxic agar plate dilution) was used originally to test fungicides. We used a herbicide (Acenit A 880 EC) instead of a fungicide when preparing the agar. On the herbicide-treated agar, Acenit was added to the medium in 1x, 2x, 5x, 10x of the regularly applied dosage per ha and we determined the colony growth of several microscopic test fungi (*Trichoderma* sp., *Fusarium oxysporum*, *Aspergillus niger*). In addition to the Acenit-treated agar, there was a control where distilled water was added to the medium. In addition to studying the growth of microscopic test fungi, we also determined the changes in the amount of the bacterium population isolated from calcareous chernozem soil in herbicide-treated agar.

The amount of carbon-dioxide released from the soil during 7 days was measured for 3 weeks to study the microbial activity of the soil (Witkamp, 1966, cit. Szegi, 1979). In one series of the examinations, only Acenit was added in different (1x, 2x, 5x, 10x per ha) dosages, while in the other series 0.5 g glucose was added per 100 g soil with which the microbial activity of soil was induced and we measured the amount of CO_2 in the soil. During the experiment the optimal moisture content was maintained (75% of the maximum water capacity).

Nitrate release in the soil was studied for four weeks and samples were taken once a week during incubation, the nitrate content of which was determined with the Na-salicylate method from a 1:5 soil-water extract (Felföldy, 1987). In addition to samples with different dosages of Acenit A 880 EC, a glucose-treated series of samples was also examined in the nitrate study. The optimum moisture content was also maintained in this experiment at 75% of the maximum water capacity.

RESULTS AND DISCUSSIONS

The growth of the studied microscopic fungi is presented in Figure 1. When evaluating the results, it can be seen that the regularly applied dosage (1x) greatly inhibited the growth of colonies in *Trichoderma* sp. and *Fusarium oxysporum*. A significant reduction was observed in the colony growth of microscopic fungi for all dosages as compared to the control. The diameter of the colonies was halved when applying the tenfold dosage. The applied herbicide dosage slightly inhibited the growth of *Fusarium oxysporum* colonies. *Trichoderma* sp. with moderate growth was sensitive to even the smaller herbicide doses.

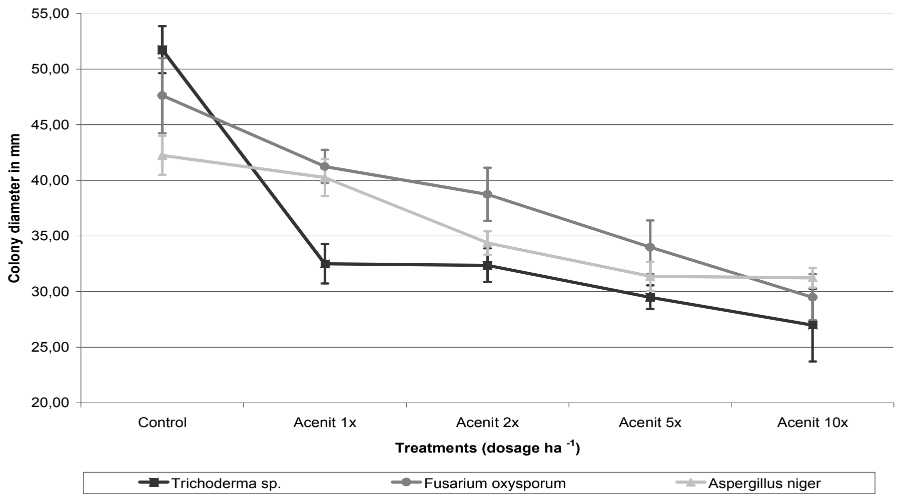
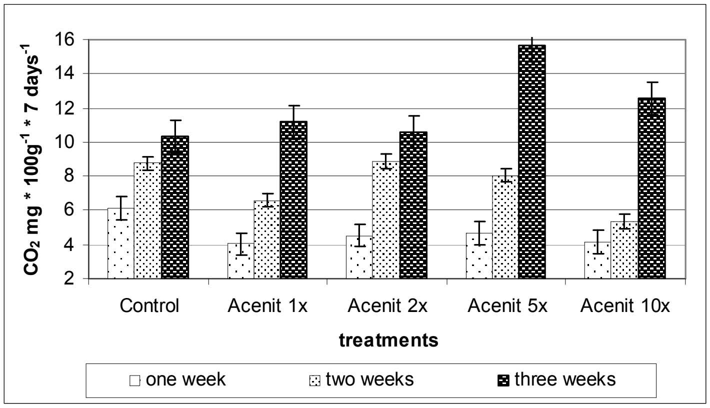
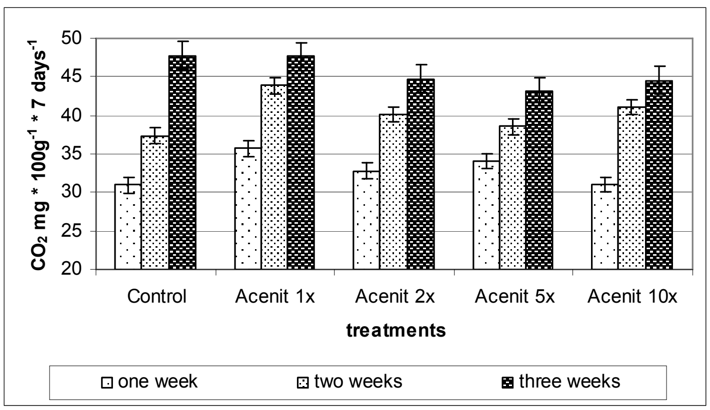


Figure 1. The effect of different dosages of Acenit A 880 EC on the colony growth of microscopic fungi (Debrecen 2005)

When studying the effect of Acenit on the population of soil bacteria, we found that the number of bacteria in the control ranged between [6.97 – 8.25] $\cdot$ 10^6 colony g^{-1} with 95% probability. In the Bouillon agar treated with herbicide, the number of bacteria indicated that the increasing concentration of herbicides did not change significantly the number of bacteria, but there was a significant reduction as compared to the control (number of bacteria: [1.90 – 2.77] $\cdot$ 10^6 colony g^{-1}). Even a low Acenit concentration greatly reduced the number of bacteria to one third of that of the control. The explanation of this can be that the herbicide killed the sensitive bacteria even at a small concentration. It can be anticipated that the resistant species remained viable even at larger concentrations.



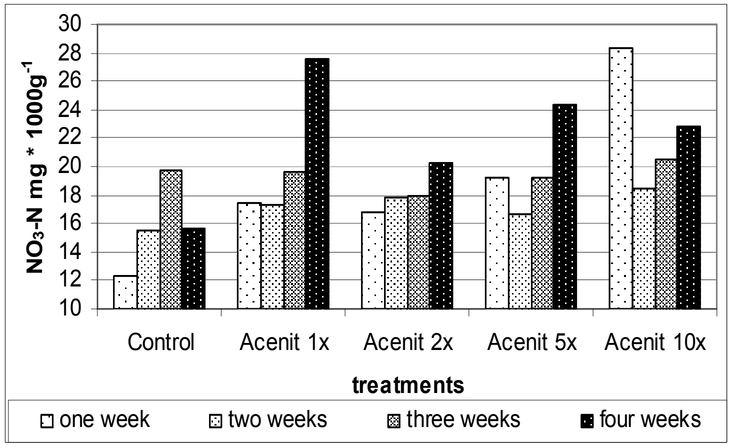
Figures 2a. The effect of Acenit A 880 EC on carbon-dioxide formation in the soil (Debrecen 2006)



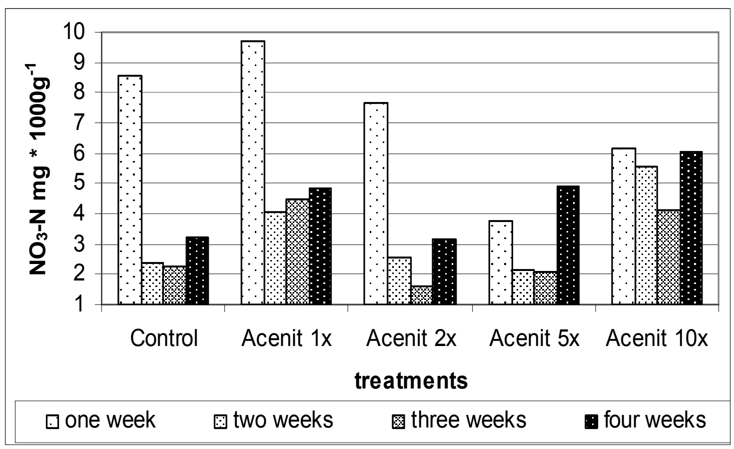
Figures 2b. The effect of Acenit A 880 EC plus glucose on carbon-dioxide formation in the soil (Debrecen 2006)

As a result of the Acenit treatment, carbon-dioxide formation in the soil reduced by the end of the first of the incubation period, and it remained lower than the control level in most of the treatments even by the end of the second week (Figure 2a). After the third week, the fivefold and tenfold dosages resulted in a considerably higher carbon-dioxide formation as compared to the control.

During the incubation period, carbon-dioxide formation increased in both series and in all treatments as well as in the control.



Figures 3a. The effect of Acenit A 880 EC on nitrate exploration (Debrecen 2006)



Figures 3b. The effect of Acenit A 880 EC plus glucose on nitrate exploration (Debrecen 2006)

As a result of the combined application of Acenit and glucose, carbon-dioxide formation in the soil (Figure 2b) was 3.5-9 times higher, as compared to the Acenit treatment alone. Glucose resulted in a significant increment in all treatments. In the first week of the incubation period, a smaller increase, then in the second week a significant increase were observed in carbon-dioxide formation, while by the end of third week a significant reduction was detected as a result of larger dosages.

In the series of samples treated with Acenit, nitrate release was higher (Figure 3a) in all treatments during the four weeks as compared to the control. A significant increase was observed at the end of the first and fourth weeks, the herbicide treatment induced nitrate formation. At the end of the second and third weeks, the differences due to the herbicide treatment were more balanced. The largest nitrate values were measured in this series. In the series treated with Acenit alone, 1.3-10 times higher nitrate values were measured.

In the samples treated with Acenit and glucose (Figure 3b), nitrate exploration decreased after the first week only by applying larger dosages. As a result of the regular and tenfold dosages, nitrate formation generally increased in the incubation period. By the end of the incubation period, the nitrate content of treated soils was higher than that of the control.

Summing up, it can be stated that microscopic fungi responded sensitively to the different herbicide dosages during their growth. A significant portion of the soil's bacterium population died after direct contact with Acenit.

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The obvious explanation of these results is that the higher organic matter content (glucose) gradually and continuously increased the carbon-dioxide producing capacity of the soil, which was less strongly influenced by Acenit. The nitrate content in the Acenit treatment was probably higher, because the microbes did not use the available soil nitrate nitrogen due to the lack of a higher amount of organic matter (substrate).

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