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# PHYTOTOXKIT$^{TM}$ MICROBIOTEST USED IN DETECTING HERBICIDE RESIDUE IN SOIL

The studies on the phytotoxic residue of herbicide active substances in the soil were conducted using a germination and early growth microbiotest Phytotoxkit$^{TM}$ by Tigret. Three plant species, *Sinapis alba, Fagopyrum esculentum* and *Cucumis sativus*, were used as bioindicators. The herbicide active substances tested included chlorsulfuron, nicosulfuron, 2,4 DP and dicamba. A plant which responded most strongly to chlorsulfuron and nicosulfuron was *Sinapis alba*. *Cucumis sativus* responded most quickly to 2,4 DP residue, while *Fagopyrum esculentum* was most suitable for the detection of dicamba residue.

## 1. INTRODUCTION

The interest in the application of plant bioassays in bioindicator testing for the residue of different xenobiotics in the soil environment has been increasing in recent years [1], [2], [3]. Tests using fast-germinating seeds have several very important advantages, as they are cheap and easy to apply, they do not require expensive laboratory equipment and these observations are easy to record and interpret as they yield reproducible results. We also need to stress the fact that some plants are sensitive to specific or very wide spectra of action of chemicals (including also herbicides) [4], [5], [6].

In ecotoxicology, bioassays are commonly applied methods to determine the rates of bioavailable phytotoxic herbicide residue in the soil [5], [7]. One of such germination and early growth microbiotests is Phytotoxkit$^{TM}$ by Tigret [8].

The aim of the study was to assess the feasibility of the application of germinating seeds of different plant species in the determination of bioavailable active substance residue of selected herbicides in the soil.

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## 2. MATERIAL AND METHODS

Investigations included four series of laboratory experiments conducted at 3-month intervals in the years 2007–2008. A microassay kit for the determination of phytotoxic herbicide residue in the soil based on germination and early plant growth, Phytotoxkit™ by Tigret, was used in the study. Three plant species are routinely used with this test: *Sorghum saccharatum*, *Sinapis alba* and *Lepidium sativum*. In order to provide information on the behaviour of other plants, Phytotoxkit™ was modified and two species, i.e. *Sorghum saccharatum* and *Lepidium sativum*, were replaced by *Fagopyrum esculentum* and *Cucumis sativus*.

Material for analyses consisted of soil samples collected from the 0–20 cm horizon, from a production field cropped to winter wheat. This soil was characterized by a slightly acid reaction ($pH_{KCl}$ = 5.5) and low organic carbon content ($C_{org.}$ = 1.42%). The soil samples collected were dried to the air moisture free level and sieved (mesh size $\phi$ = 2 mm). Sieved soil was placed successively on testing plates and soaked with distilled water until a total water capacity was obtained. Active substance residue was analyzed for the following herbicides: Glean 75 WG (chlorsulfuron), Milagro 040 SC (nicosulfuron), Aminopielik Plus 570 SL (2,4 DP) and Banvel 480 SL (dicamba) within the range of 0.025–1.2 mg/kg. Testing plates were placed in an Aporo spraying chamber, where herbicides were applied in amounts ensuring the assumed concentrations of the active substances in soil samples. Next plates were covered with filter paper and seeds of the test plants, i.e. *Sinapis alba, Fagopyrum esculentum* and *Cucumis sativus*, were sown at a rate of 5 seeds per plate. Test plates were incubated in the vertical position at 25 °C in the dark for the period of 5 days. The response of plants to the presence of chlorsulfuron, nicosulfuron, 2,4 DP and dicamba was assessed on the basis of root length reduction in relation to the control (not sprayed with a herbicide). Images were recorded using a digital camera, and Image Tools image analysis software was used in measurements of root length in test plants. The results were subjected to the analysis of variance, comparing the significance of differences at $\alpha \leq 0.05$. The microbiotest procedure was described in detail in the standard procedure [8].

## 3. RESULTS AND DISCUSSION

According to DEMCZUK et al. [4], SADOWSKI et al. [5], HERNÁNDEZ-SEVILIANO et al. [9] and KLIMKOWICZ-PAWLAS et al. [10] the selection of an appropriate test plant, being in an adequate development phase and responding to the xenobiotic tested, is the primary and most difficult element affecting the bioassay accuracy.

Figures 1–4 present the results of analyses for Phytotoxkit™ using the effect of root length reduction in *Sinapis alba, Fagopyrum esculentum* and *Cucumis sativus* when determining concentrations of herbicide active substance residue in the soil.

The plant exhibiting the strongest response to chlorsulfuron and nicosulfuron was *Sinapis alba,* followed by *Fagopyrum esculentum*, while the weakest response was recorded for *Cucumis sativus*. The dependence of root length reduction on the concentrations, ranging from the lowest (0.025 mg/kg) to the highest (1.2 mg/kg), of chlorsulfuron and nicosulfuron in the soil showed that the plants selected, i.e. *Sinapis alba* and *Fagopyrum esculentum*, responded most strongly to these substances. The detoxication capacity ($ED_{50}$) for the above mentioned substances in these plants was neutralized at as low concentration as 0.07 mg/kg (chlorsulfuron) and 0.125 mg/kg (nicosulfuron), while a further increase in the concentrations of these substances resulted in a strong reduction of root length (figures 1–2).

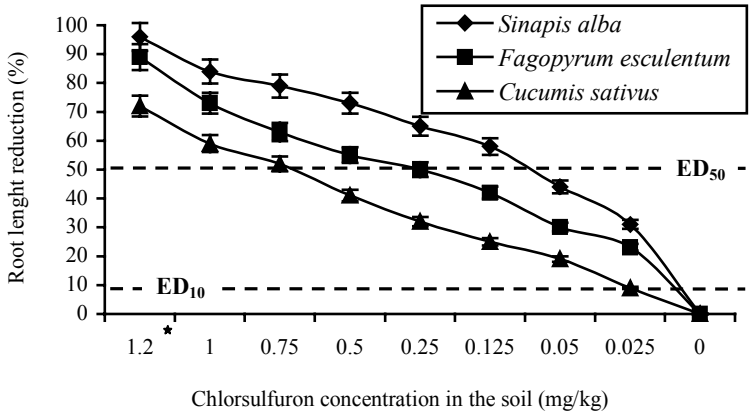


Fig. 1. Effect of chlorsulfuron on plant root length reduction (* – non-significant differences at $\alpha \leq 0.05$)

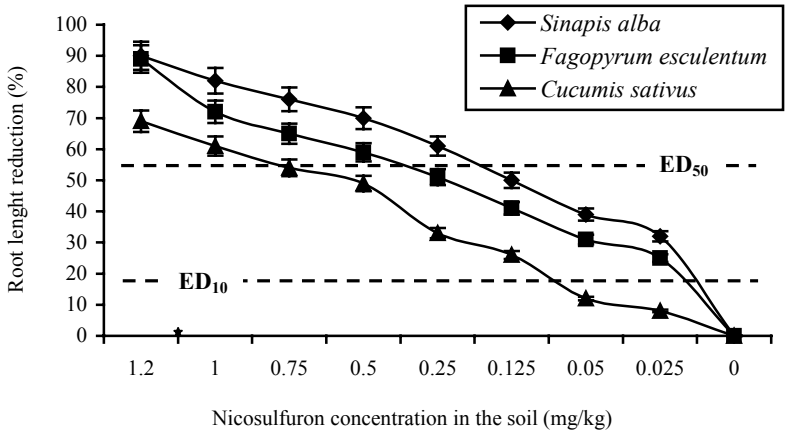


Fig. 2. Nicosulfuron effect on plant root length reduction (* – non-significant differences at $\alpha \leq 0.05$)

Similar results were also reported by SADOWSKI et al. [5] in their studies on the detection and behaviour of sulfsulfuron and chlorsulfuron residue in the soil with the use of a conventional bioassay.

The weakest response to chlorsulfuron and nicosulfuron was found for *Cucumis sativus.* In the case of this plant, $ED_{50}$ for chlorsulfuron and for nicosulfuron was 0.75 mg/kg and 0.6 mg/kg, respectively (figures 1–2). The weak response of this plant limits its suitability for the detection of slight concentrations of the substances analyzed. Also in their earlier studies SEKUTOWSKI & SADOWSKI [11] indicated a relatively poor sensitivity of *Cucumis sativus* to thifensulfuron-methyl.

For 2,4 DP this gradation is different: *Cucumis sativus* exhibited the strongest response, followed by *Fagopyrum esculentum*, while *Sinapis alba* ranked last (figure 3).

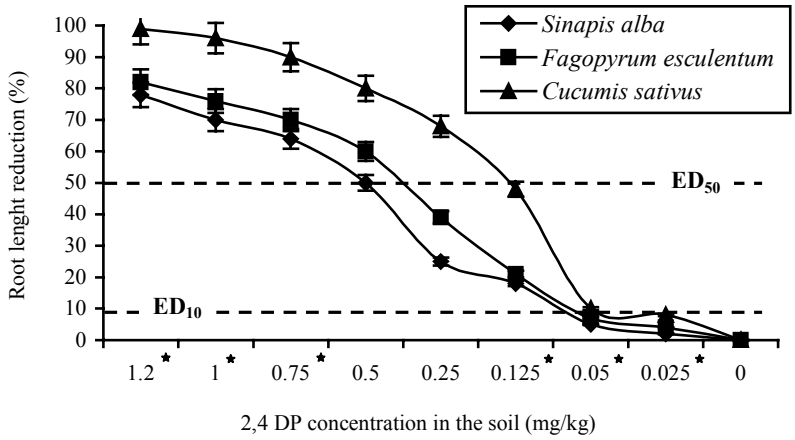


Fig. 3. Effect of 2,4 DP on plant root length reduction
(* – non-significant differences at $\alpha \leq 0.05$)

Response of *Cucumis sativus, Fagopyrum esculentum* and *Sinapis alba* to 2,4 DP at the lowest concentrations ($ED_{10}$) was statistically non-significant. With an increase in 2,4 DP concentration the differences between the plants analyzed changed and at the highest concentration the root length in *Cucumis sativus* was reduced by 99% and in *Fagopyrum esculentum* and *Sinapis alba*, by approx. 80% (figure 3). It may be clearly observed that the capacity of *Cucumis sativus* ($ED_{50}$) to detoxicate 2,4 DP was neutralized at as low its concentration as 0.125 mg/kg. Such a strong response of *Cucumis sativus* makes it possible to analyze even slight residue of 2,4 DP in the soil.

When determining the phytotoxicity thresholds for dicamba it was found that significant differences in root length reduction in *Cucumis sativus* and *Fagopyrum esculentum* were observed for concentrations ranging from 0.025 mg/kg to 0.25 mg/kg. However, the strongest response to this substance was recorded for *Fagopyrum*

*esculentum*. The capacity of *Fagopyrum esculentum* ($ED_{50}$) to detoxicate dicamba was neutralized at as low its concentration as 0.125 mg/kg, while a further increase in the concentration of this substance in the soil (1.2 mg/kg) caused root length reduction by 99% (figure 4). Similar results were recorded by SADOWSKI et al. [5] and WALL & SMITH [12] when comparing the effect of chlorsulfuron, pendimethalin, chlortoluron, MCPA, 2,4 D and 2,4 DP on dry matter reduction in selected test plants.

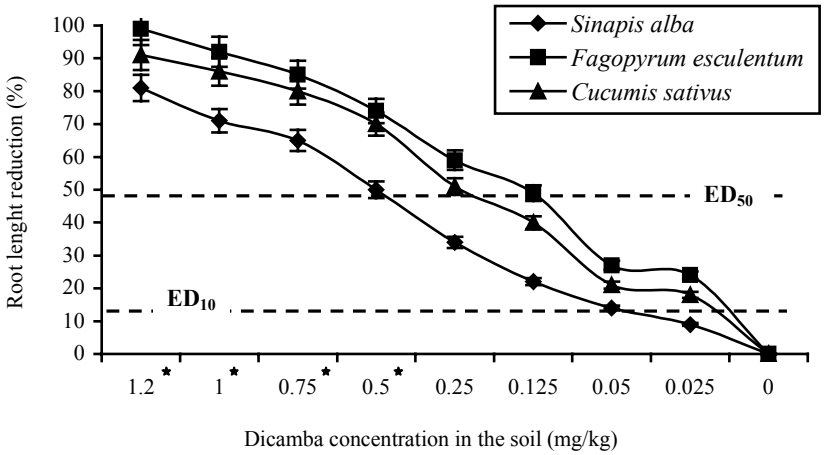


Fig. 4. Effect of dicamba on plant root length reduction
(* – non-significant differences at $\alpha \leq 0.05$)

## 4. CONCLUSIONS

1. *Sinapis alba* turned out to be most suitable for detecting chlorsulfuron and nicosulfuron residue in the soil, followed by *Fagopyrum esculentum,* while the weakest response to the substances analyzed was observed in *Cucumis sativus*.

2. In the case of 2,4 DP residue in the soil, *Cucumis sativus* proved to be the most sensitive plant. The other phytodetectors, i.e. *Fagopyrum esculentum* and *Sinapis alba*, responded much weakly to the substance analyzed.

3. *Fagopyrum esculentum* proved to be most suitable for the detection of dicamba residue. A slightly weaker response was observed for *Cucumis sativus,* while the weakest response to this substance was recorded for *Sinapis alba.*

4. In the studies with plants used as detectors, the selection of an appropriate plant in order to test herbicide active substance is of a crucial importance. A sufficiently sensitive plant makes it possible to conduct analyses at a residue concentration of 0.0015 mg/kg soil.

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## WYKORZYSTANIE TESTU PHYTOTOXKIT™ W WYKRYWANIU POZOSTAŁOŚCI HERBICYDÓW W GLEBIE

W badaniach fitotoksycznych pozostałości substancji aktywnych herbicydów w glebie wykorzystano zestaw do kiełkowania i wczesnego wzrostu roślin Phytotoxkit™ firmy Tigret. Jako bioindykatory stosowano 3 gatunki roślin: *Sinapis alba, Fagopyrum esculentum* i *Cucumis sativus*. Badanymi substancjami aktywnymi herbicydów były: chlorosulfuron, nikosulfuron, 2,4 DP oraz dikamba. Rośliną, która najsilniej reagowała na chlorosulfuron i nikosulfuron, była *Sinapis alba*. Na pozostałości 2,4 DP najszybciej reagował *Cucumis sativus*, natomiast *Fagopyrum esculentum* najlepiej nadawał się do wykrywania pozostałości dikamby.