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Microbial Toxicity of Pesticide Derivatives Produced with UV-photodegradation

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Abstract Our study aimed at acquiring information about the biological effect of pesticides and their degradates produced by UV-treatment on microbiological activity. Five photosensitive pesticides (carbendazim, acetochlor, simazine, chlorpyrifos, EPTC) and six representative soil microbes (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Mycobacterium phlei*, *Fusarium oxysporum*, *Penicillium expansum*, *Trichoderma harzianum*) were applied throughout our model experiments. The antimicrobial effects of the pesticides and their degradates were assessed with filter paper disk method. The antimicrobial effect of the degradation products exhibited marked differences in terms of pesticide types, irradiation time, and the test organisms. Acetochlor and its photolytic degradation products were found to be more toxic to bacteria than fungi. All the three bacteria proved to be sensitive to the basic compound and its degradation products as well. The end product of carbendazim was weakly antibacterial against *P. fluorescens* and *B. subtilis* but strongly antifungal against *T. harzianum*. Chlorpyrifos and its end product inhibited neither test organisms, but the degradates hindered the growth of four of them. The basic compound of EPTC and the degradates of simazine exhibited significant toxicity to the test bacteria. It might be claimed that the pesticide photodegradation may result in significant changes in soil microbiota, as well as formation of biologically harmful degradates.

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Pesticides sprayed on the soil surface are exposed to effect of UV photons resulting in decomposition of the molecule. The toxicity of the given pesticides is investigated during their registration process, but the toxicity of the degradation products to the soil microorganisms is unexplored yet. Only a few reports dealing with the photodegradation mechanisms of pesticides provide data about the hazards of the degradates (Burrows et al. 2002). Even in these cases, standard genotoxicity tests using *Salmonella bacterium* (Bartos et al. 2005), environmental toxicity tests with *Vibrio fisheri* (e.g. Bonnemoy et al. 2004; Dimou et al. 2005) were applied or aquatic organisms were used as test organisms (Iesce et al. 2004). Although soil microorganisms have more direct exposition to the photoproducts of the pesticides they were omitted from toxicity investigations.

The objective of our work was to acquire information about the microbiological activity of the basic compounds and the degradates of five photosensitive pesticides of different type and behaviour. Six representative soil microbes were applied as test organisms throughout our microbiological model experiments.

Materials and Methods

Five photosensitive pesticides of diverse chemical structures and reaction mechanisms were exposed to UV-light by means of a special immersible instrument emitting light of 254 nm (Kiss et al. 2007). Analytical grade carbendazim

(benzimidazole fungicide), acetochlor (chloracetanilide herbicide), simazine (triazine herbicide), EPTC (thiocarbamate herbicide), and chlorpyrifos (organophosporus insecticide) were purchased from Aldrich. The degradation process was followed by thin-layer chromatography and gas chromatography technique until total phototransformation of the basic compounds.

Three bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Mycobacterium phlei*) and three filamentous fungus species (*Fusarium oxysporum*, *Penicillium expansum*, *Trichoderma harzianum*) were applied as test organisms. The antimicrobial effects of the pesticides and their degradates were assessed with filter paper disk method. Standard Whatman No.1. disks of 5 mm diam. were filled with 100 µl aliquots of the reaction mixture containing 50 µg soluble dry material. Surface of Nutrient agar and Malt-extract agar was inoculated with the cell suspension of bacterial and fungal strains, respectively. After drying four disks were placed on the surface of the inoculated substrates. The size of the growth inhibitory zone was measured after 48 h of incubation at 30°C in the dark. The test was performed in triplicates. Data were averaged and the standard error was calculated with Statgraphics 5.0 software.

Results and Discussion

The antimicrobial effect of the degradation products exhibited marked differences in terms of pesticide types, irradiation time, and the test organisms (Tables 1, 2). Acetochlor and its photolytic degradation products were found to be more toxic to bacteria than fungi. Zheng et al. (2003) also established that the application of acetochlor enhanced the growth of the studied soil fungi. The antibacterial activity of degradates of acetochlor gradually increased as a function of irradiation time. All the three bacteria proved to be sensitive to the basic compound and its degradation products as well. Among them *M. phlei* displayed the most pronounced sensitivity. Measurable antifungal activity was detected just after total transformation of the basic compound. Liu et al. (2005) suggested that the UV-degradation of this pesticide might result in the formation of a series of new compounds. It should be clarified in further studies whether the end products have a general antimicrobial effect or both antibacterial and antifungal character might be observed.

The degradation products of fungicide carbendazim exerted no effect on test bacteria and on fungus *P. expansum*. However, significant temporary influence of the pesticide was observed after 10 h of irradiation in the case of *T. harzianum*, and decreasing inhibition was detected in *F. oxysporum* colonies. The end product was weakly antibacterial against *P. fluorescens* and *B. subtilis* but strongly antifungal against *T. harzianum*.

On the contrary, the insecticide chlorpyrifos and its end product inhibited neither test organisms, but the degradates hindered the growth of four of them. Growth

Table 1 Antibacterial effect of pesticides and their degradates after 48 h incubation time

Pesticides	UV irradiation time (min)	Inhibitory zone of degradation mixes (mm)		
		B. subtilis	*M. phlei*	*P. fluorescens*
Acetochlor	0	0.0 *a*	1.0 *b*	2.3 *bc*
	0.5	0.0 *a*	2.0 *bc*	1.6 *b*
	1	1.0 *b*	2.6 *bc*	2.6 *bc*
	2	1.0 *b*	3.6 *c*	1.6 *b*
	5	1.0 *b*	8.0 *d*	3.6 *c*
	10	2.0 *bc*	5.0 *d*	4.3 *cd*
	16	3.0 *c*	9.6 *f*	7.3 *e*
Carbendazim	0	0.0 *a*	0.0 *a*	0.0 *a*
	0.5	0.0 *a*	0.0 *a*	0.0 *a*
	1	0.0 *a*	0.0 *a*	0.0 *a*
	2	0.0 *a*	0.0 *a*	0.0 *a*
	5	0.0 *a*	0.0 *a*	0.0 *a*
	10	0.0 *a*	0.0 *a*	0.0 *a*
	13	0.0 *a*	0.0 *a*	0.0 *a*
	20	1.0 *b*	0.0 *a*	1.0 *b*
Chlorpyrifos	0	0.0 *a*	0.0 *a*	0.0 *a*
	0.5	0.0 *a*	0.0 *a*	0.0 *a*
	1	0.0 *a*	0.0 *a*	0.0 *a*
	2	1.0 *b*	0.0 *a*	1.0 *b*
	5	1.0 *b*	1.0 *b*	1.0 *b*
	11	+	1.6 *b*	+
	16	0.0 *a*	1.0 *b*	0.0 *a*
	30	0.0 *a*	0.0 *a*	0.0 *a*
EPTC	0	5.0 *c*	4.6 *c*	7.0 *d*
	0.5	+	0.0 *a*	0.0 *a*
	1	1.3 *b*	0.0 *a*	0.0 *a*
	2	0.0 *a*	0.0 *a*	0.0 *a*
	5	0.0 *a*	0.0 *a*	0.0 *a*
	10	0.0 *a*	0.0 *a*	0.0 *a*
	15	0.0 *a*	0.0 *a*	0.0 *a*
Simazine	0	0.0 *a*	1.3 *b*	0.0 *a*
	0.5	+	1.0 *b*	0.0 *a*
	1	+	+	0.0 *a*
	2	0.0 *a*	2.3 *b*	0.0 *a*
	5	0.0 *a*	2.0 *b*	0.0 *a*
	10	+	9.0 *e*	4.6 *c*
	12	1.0 *b*	9.6 *e*	6.0 *d*

Values are means of three replicates. Differing letters mark significantly different ($p < 0.05$) values for a given pesticide. Plus sign (+) marks visible, but not measurable (<1 mm) inhibition zones

Table 2 Antifungal effect of pesticides and their degradates after 48 h incubation time

Pesticide	UV irradiation time (min)	Inhibitory zone of degradation mixes (mm)		
		F. oxysporum	*P. expansum*	*T. harzianum*
Acetochlor	0	0.0 *a*	0.0 *a*	0.0 *a*
	0.5	0.0 *a*	0.0 *a*	0.0 *a*
	1	0.0 *a*	0.0 *a*	0.0 *a*
	2	0.0 *a*	0.0 *a*	0.0 *a*
	5	0.0 *a*	+	0.0 *a*
	10	0.0 *a*	1.3 *b*	0.0 *a*
	16	3.0 *c*	7.0 *d*	3.0 *c*
Carbendazim	0	10.0 *c*	0.0 *a*	24.0 *f*
	0.5	7.3 *b*	0.0 *a*	0.0 *a*
	1	7.3 *b*	0.0 *a*	0.0 *a*
	2	6.6 *b*	0.0 *a*	0.0 *a*
	5	7.0 *b*	0.0 *a*	0.0 *a*
	10	7.0 *b*	0.0 *a*	20.0 *e*
	13	7.0 *b*	0.0 *a*	0.0 *a*
	20	0.0 *a*	0.0 *a*	14.0 *d*
Chlorpyrifos	0	0.0 *a*	0.0 *a*	0.0 *a*
	0.5	0.0 *a*	1.0 *b*	0.0 *a*
	1	0.0 *a*	13.3 *d*	0.0 *a*
	2	0.0 *a*	1.3 *b*	0.0 *a*
	5	0.0 *a*	+	0.0 *a*
	11	0.0 *a*	4.0 *c*	0.0 *a*
	16	0.0 *a*	1.3 *b*	0.0 *a*
	30	0.0 *a*	0.0 *a*	0.0 *a*
EPTC	0	0.0 *a*	6.3 *b*	0.0 *a*
	0.5	0.0 *a*	0.0 *a*	0.0 *a*
	1	0.0 *a*	0.6 *a*	0.0 *a*
	2	0.0 *a*	0.0 *a*	0.0 *a*
	5	0.0 *a*	0.0 *a*	0.0 *a*
	10	0.0 *a*	11.3 *c*	0.0 *a*
	15	0.0 *a*	21.0 *d*	0.0 *a*
Simazine	0	0.0 *a*	0.0 *a*	0.0 *a*
	0.5	0.0 *a*	0.0 *a*	0.0 *a*
	1	0.0 *a*	0.0 *a*	0.0 *a*
	2	0.0 *a*	0.0 *a*	0.0 *a*
	5	0.0 *a*	0.0 *a*	0.0 *a*
	10	0.0 *a*	+	0.0 *a*
	12	+	0.0 *a*	0.0 *a*

Values are means of three replicates. Differing letters mark significantly different ($p < 0.05$) values for a given pesticide. Plus sign (+) marks visible but not measurable (<1 mm) inhibition zones

of *B. subtilis* and *P. fluorescens* bacteria was inhibited by the degradates formed subsequent to 2 and 5 h of UV-irradiation, while *M. phlei* was sensitive to the products generated after 5–16 h long UV-treatment. Among the test fungi, *T. harzianum* and *F. oxysporum* did not show sensitivity towards to the basic compound and its degradation products, whereas *P. expansum* was sensitive to all of the degradates. The highest inhibition effect was attributed to the degradates obtained after 1 h-long irradiation. It decreased after 2 h, and increased again after 11 h of irradiation.

EPTC was toxic to the all three bacteria studied, but this effect decreased after half or 1 h of UV-treatment. Similarly to chlorpyrifos, only *P. expansum* was proved to be susceptible to pesticide degradates among the fungi. It was inhibited moderately by the basic compound, weakly by the degradation products, and strongly by the end product. *T. harzianum* and *F. oxysporum* exhibited sensitivity neither to the basic compound nor to its degradation products. This result was in contrast with findings of Marco and Hayes (1979), who noted that the degradation products had higher biotoxicity than the basic compound. This might be explained by the fact that different test-organisms have been applied throughout the two studies.

Contrary to the findings of Gaur and Misra (1978), simazine proved to be more toxic to the test bacteria than to fungi. The degradation products of simazine had significant toxicity only to the test bacteria. The size of inhibition zone increased as a function of UV-treatment time, and reached the maximum in case of the sample obtained after 16 h of UV-irradiation. *M. phlei* was found to be the most sensitive bacterium, and this behaviour was more pronounced in case of the degradates. *P. fluorescens* was affected by the products obtained after 10 h of UV-treatment, while the growth of *B. subtilis* was inhibited only by the final degradation products. Simazine and its derivatives had no effect on the growth of the test fungi, but in certain cases they blocked or deformed the sporulation.

Summarizing the results, the toxicity of UV-degradation products of the tested pesticides showed significant variability in terms of both the basic compound and the test organisms. Chlorpyrifos was the only pesticide, which had only temporary toxicity to some soil microbes. EPTC exhibited similar characteristics, as proved to be toxic only to the test fungus *P. expansum*. Three test organisms were inhibited by the final degradation products of carbendazim and simazine, however, the latter was deemed as a pesticide of higher risk because of its broad and increasing antibacterial effect. Acetochlor appeared to entail the highest environmental risk since its end products may have a broad inhibitory spectrum against various soil microorganisms. To sum up the abovementioned findings we may claim that the pesticide photodegradation may result in significant changes in soil microbiota, as well as formation of biologically harmful degradates. Toxicity tests of the pesticide degradation products with natural soil samples

will be performed to confirm our findings under conditions complying with the natural environment.

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