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DISTRIBUTION OF MICROSCOPIC FUNGI DECOMPOSING SOME HERBICIDES IN SOILS OF BULGARIA

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Microscopic fungi are characterized by a strong fermentation system and widely spread mycelia in the soil. In particular, they determine to a great extent the biological activity of acid soils. Soil fungi decompose herbicides as well [7] but the studies in that respect are still very scarce [3, 10]. The data about their distribution in soils and their enzyme activity, as regards decomposition of some herbicides, will contribute to the clasification of certain details in the process of the biological detoxication of the preparations and the determination of the measures aiming at controlling their toxic accumulation in the soil.

METHODS

Microscopic fungi have been isolated from different soils repeatedly in the course of different seasons: chernozems (typical, leached, podzolized), cinnamonic (typical, leached, podzolized), brown and grey forest soils, humic calcareous, deluvial-alluvial-meadow and smolnitsa soils. Isolation has been achieved by the plate method of a diluted soil suspension in the K a u f m a n 's medium [8] containing 5 mg/l of each of the triazine herbicides: simazine and atrazine, as well as the amide ones; alidochlor (CDAA), alachlor and propachlor as a source of carbon. Identical investigations on the same medium, but without herbicides (with sugar as a carbon source) have been carried out as controls.

Ectoenzymes of the culture solution have been purified from the definite species of microscopic soil fungi after a 10-day incubation of the microorganisms on the K a u f m a n's medium [8]. Purification of enzymes has been made by dialysis. The decomposition of herbicides has been established after a 2-hour effect of enzymes according to I w a z a k i [5].

RESULTS

Simazine and atrazine decreased to a little extent the number of microscopic fungi (Fig. 1 and 2). This slight effect of triazine herbicides on the growth of soil microorganisms could be explained by their low toxicity [9]. The number of microscopic fungi tested in the two variants was rather similar, but when atrazine was used the number of fungi adapted to this herbicide and capable to decompose it, was smaller.

The amide-herbicide alidochlor appeared to be most toxic (Fig. 1 and 2). According to L j u b i e n o v [9] the doses LD_{50} of alidochlor was

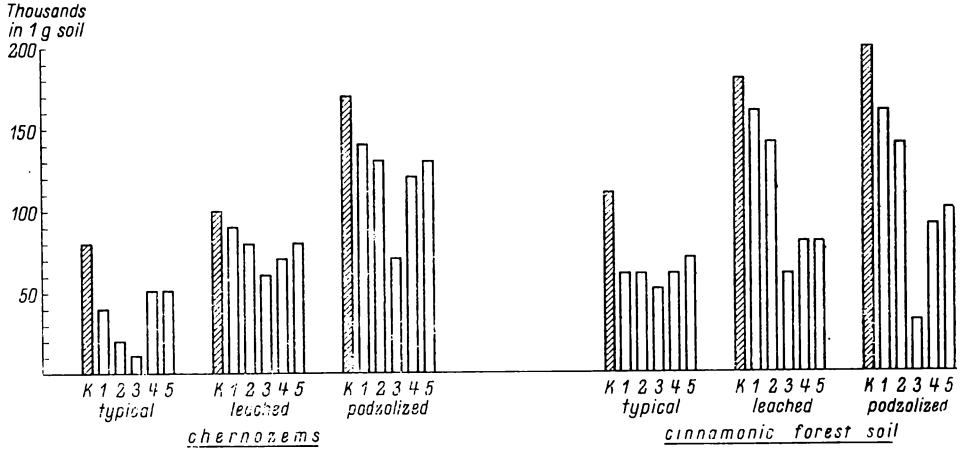

Fig. 1. Microscopic fungi of chernozem and cinnamonic forest soils on herbicide media

K — control, *1* — simazine, *2* — atrazine, *3* — alidochlor (CDAA), *4* — alachlor, *5* — propachlor

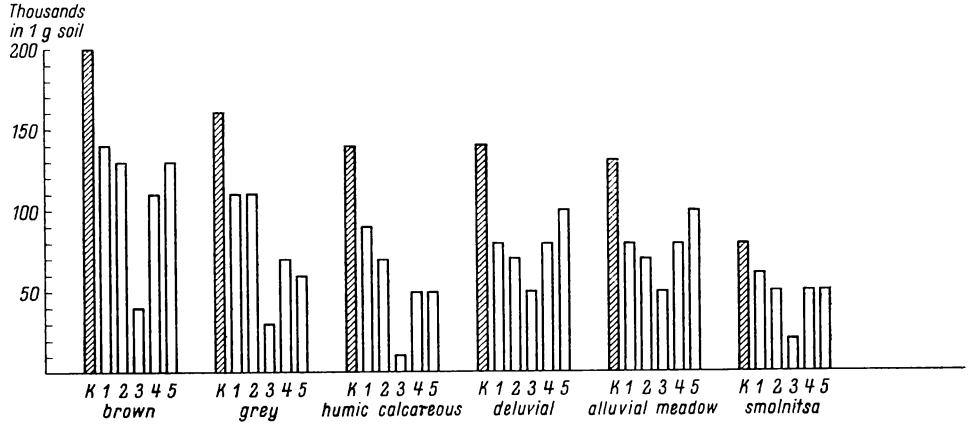

Fig. 2. Microscopic fungi of brown, grey and other soils on herbicide media

K — control, *1* — simazine, *2* — atrazine, *3* — alidochlor *4* — alachlor, *5* — propachlor

700 mg/kg, while LD_{50} of alachlor and parachlor, atrazine, simazine was 1200, 3080 and 5000 mg/kg, respectively. These data explained the various effect of the used herbicides on soil microflora.

The number of the microscopic fungi in the different soils depended on the population of those microorganisms. It was found that in the brown and grey forest soil, podzolized chernozem and cinnamonic forest soil, the number of fungi decomposing alidochlor was 90 thousand/g, while in the smolnitza, typical chernozem and humic calcareous soil it was considerably smaller (10 thausand/g). This fluctuation of the fungal number in different soils could be used as a base for the herbicides application in agriculture and, on the other hand, to indicate the danger of the herbicide accumulation in the soils and their toxicity.

Two amide herbicides alachlor and propachlor appeared to be less toxic. It is possible that these herbicides could be a source of carbon for many microscopic fungi. Toxicity of alachlor and propachlor varied depending on the type of soils. In chernozem and the brown forest soil this two amide herbicides had no effect on the number of fungi while in the leached podzolized cinnamonic, grey forest and humic calcareous soils, the herbicides decreased the number of fungi by about 50%.

The genetic composition of microscopic soil fungi in the media with the herbicides was different from that of the control medium (Table 1). In the media with amide-herbicides the genus *Penicillium* was predominant, while in the media with triazines the number of *Penicillium* genus was decreased and other genera of fungi appeared. This prevalence of *Penicillium* genus could be explained by their usually great number in the soil [2] and by their strong fermentation system and ability to decompose the herbicides in soils [3]. The strong enzymatic activity of the *Penicillium* genus could be a reason for their adaptation to the used herbicides, or even for the decomposition of some herbicides, egg. alidochlor (Table 2).

The fungi of genus *Aspergillus* does not occur in a great number in our soils [2]. They have been also found in studied soils in smaller number than *Penicillium* (Table 1), especially in the medium with alachlor and parachlor.

The microscopic fungi of the genera *Trichoderma* and *Fusarium* have been found in Bulgarian soils, but rather in a low number [2]. Microscopic soil fungi of the genus Mucor and others have been isolated from some of the soils only and on the media with triazine herbicides (Table 1).

The determination of the ability of fungi to decompose alichlor showed that 10 species of fungi could do it to the various extent (Table 2). The most active was *Fusarium oxysporum, Fusarium moniliforme*, then some species of *Penicillium* and *Fusarium* (8-13%). The lowest activity

was shown by *Penicillium funiculosum* (3%) and *Aspergillus foncecaeus* 2%.

The establishment of the alidochlor decomposition by some soil fungi indicates a possibility of biodegradation; on the other hand, the introduction of alidochlor into the soil with low biodynamic activity should be cautious. It was found that the other herbicides were also decomposed

Table 1

Generic composition of populations of soil microscopic fungi, developing on nutrient media with herbicides, %

Soil	Herbicide	Penicillium	Aspergillus	Trichoderma	Fusarium	Mucor	Others
Smolnitsa	nil	28.6	2.0	14.3	27.1	-	10
	simazine	43.0	14.2	28.6	14.2	-	-
Isozhourishtel	atrazine	30.0	40	10	20	-	-
	alidochlor	100	-	-	-	-	-
	propachlor	75.0	25	-	-	-	-
	alachlor	85.7	14.3	-	-	-	-
Leached chernozem	nil	20	2.0	-	10	-	40
	simazine	28.5	14.3	-	14.3	-	48.9
I Gen.Toshevol	atrazine	33.3	16.7	-	-	-	50
	alidochlor	100	-	-	-	-	-
	propachlor	50	-	-	50	-	-
	alachlor	60	20	-	-	-	20
Typical cynnamonic	nil	43.8	12.5	12.5	13	-	48.2
	simazine	63.6	18.2	18.2	-	-	-
/Slivnitsa/	atrazine	55.6	11.1	22.2	-	-	-
	alidochlor	100	-	-	-	-	-
	propachlor	61.5	15.4	7.70	-	-	15.4
	alachlor	77.6	-	11.1	-	-	12.1
Grey forest	nil	40	20	-	20	-	20
/Nikolayevo/	simazine	66.4	-	-	33.6	-	-
	atrazine	50	-	-	50	-	-
	alidochlor	100	-	-	-	-	-
	propachlor	100	-	-	-	-	-
	alachlor	100	-	-	-	-	-
Brown forest	nil	51.4	3	5	15.3	-	15.3
/Borovests/	simazine	66.4	22.5	-	11.1	-	-
	atrazine	61.6	30.6	7.6	-	-	-
	alidochlor	100	-	-	-	-	-
	propachlor	100	-	-	-	-	-
	alachlor	75	-	-	2.5	-	-
Humic calcareous	nil	36	8	8	24	20	4
/Chirpan/	simazine	47	-	-	23.5	13.7	17.8
	atrazine	40	-	-	30	30	-
	alidochlor	100	-	-	-	-	-
	propachlor	50	-	50	-	-	-
	alachlor	74.4	-	-	28.6	-	-

by soil fungi to different degree, depending on fungi species and chemical structure of the preparations.

Table 2

Decomposition of alidochlor by the enzymes of some microscopic fungi

Species of microscopic fungi	Per cent of decomposed preparation
Fusarium oxysporum	13
Fusarium moniliforme	40
Fusarium solani	9
Fusarium sambicinum	8
Tnichoderma viride	7
Penicillum frequentans	12
Penicillum lilacinum	8
Penicillum funiculosum	3
Aspergillus flavus	6
Aspergillus fonceceus	2

CONCLUSIONS

1. The number of soil microscopic fungi isolated on media of the herbicides used, depends on the type of soil and herbicides.

2. The amide herbicides (alachlor, propachlor and alidochlor in particular) exert an inhibitory effect on number of the fungi. The triazine herbicides (atrazine and simazine) are characterized by a weak effect in this respect.

3. The generic composition of the populations of microscopic soil fungi varies depending on the type of the herbicide used. *Penicillium* is prevailing, especially when the preparations used are more toxic.

4. The ectoenzymes of pure cultures of some microscopic fungi decompose the herbicides as regards species of microorganisms.

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ROZMIESZCZENIE W GLEBACH BUŁGARII GRZYBÓW MIKROSKOPOWYCH ROZKŁADAJĄCYCH NIEKTÓRE HERBICYDY

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Streszczenie

Badano rozmieszczenie grzybów mikroskopowych rozkładających herbicydy: CDAA, alachlor, propachlor, simazynę i atrazinę. Stwierdzono zależność między występowaniem różnych gatunków grzybów a rodzajem gleby i obecnością herbicydów. Herbicydy amidowe wpływały hamująco na wzrost grzybów, natomiast triazynowe wykazywały tylko nieznaczne działanie. Określono zdolność do rozkładu herbicydów przez czyste kultury poszczególnych gatunków grzybów.

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РАСПРЕДЕЛЕНИЕ МИКРОСКОПИЧЕСКИХ ГРИБОВ РАЗЛАГАЮЩИХ НЕКОТОРЫЕ ГЕРБИЦИДЫ В ПОЧВАХ БОЛГАРИИ

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Резюме

Автор исследовал качественное распределение микроскопических грибов разлагающих гербициды: CDAA, алахлор, пропахлор, симазин и атразин.

Обнаружена зависимость между появлением разных видов грибов, видом почвы и применяемыми гербицидами. Гербициды аминной группы задерживали рост грибов, тогда как триазинные гербициды обнаруживали только незначительное действие. Определяли способность разложения гербицидов чистыми культурами отдельных видов грибов.