

## Prokaryotes and eukaryotes microorganisms type with some important agricultural chemical resistant

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### Abstract

In the present work, our goal was to establish the resistance of microscopic prokaryotes and eukaryotes on three sulfonylurea substances in soil or prepared as suspensions. Based on obtained data, different kind of bacteria, actinomycetes, filamentous fungi and algae were observed. Among the species of bacteria, we mention the *Bacillus megaterium*. As actinomycetes, there are the following: *Streptomyces griseus*, *Streptomyces albus*, *Streptomyces aureus*. *Penicillium* and *Rhizopus* are mold species. *Coccomyxa lacustris* has been taken out from the Green algae group.

**Keywords:** Glean, Grodyl, Harmony, *Bacillus megaterium*, *Streptomyces griseus*, *Penicillium*.

### 1. Introduction

Herbicides, as a stressful problem of the microbial population, have become one of the much discussed issues. The toughest challenge in this area is related to criteria for assessing the aggressiveness of herbicides on the micro flora. *Actinomycetes*, bacteria and fungi have a different kind of sensitivity to most herbicides. Their sensitivity is also influenced by other factors. Usually, aerobe and facultative aerobe microorganisms are more sensitive than anaerobic microorganisms. *Streptomyces* have a better tolerance than fungi. Bacteria that form spores are often more sensitive than those who do not form spores.

It is said that soil bacteria generally has second effects in the presence of herbicides, but data from specialized literature are often contradictory [2,3]. Bacteria that form endospores are more resistant than vegetative cells. Resistance increases with age.

Organisms living freely in soil, compared to those associated with other organisms, are more sensitive to chemical toxicity [5,6]. Algae are severely inhibited by inhibiting herbicide photosynthesis, even at low doses by type of substance and species [8,9].

Because of the sensitivity to this group of herbicides, some kind of green algae (*Chlorella*) are used for bio tests with residual herbicides [10]. Chlorsulfuron - has been much studied in the world [4,7].

A way of acting has been settled with its penetration in the plant, causes selectivity, field of use, application parameters, the spectrum of action, possibilities of combination with other herbicides, and the potential residual effect.

Dosage problem was the subject of extensive concern [8] as well as for products with high selectivity. Amidosulfuron has the same environmental effect as the Chlorsulfuron [1].

## 2. Materials and methods

Three phyto pharmaceutical substances from the sulphonylurea group and these are: Glean, and Harmony Grodyl. Those three substances were used to obtain the suspension-herbicides, used to treat life in the environment, meaning soil microorganisms. Herbicide concentrations were determined according to the quantity of treated soil in laboratory models or depending on plot size from the field.

Tests begun with doses approved for agricultural practices (Glean 20 g/ha Grodyl 60g/ha, Harmony 60 g/ha). Doses were increased two or five times (Glean 40 and 100g/ha, Grodyl 120 and 300g/ha, Harmony 120 and 300g/ha). Microorganism isolation has been performed after 7 days of treatment in laboratory conditions and 30 days of treatment applied under natural conditions. For bacteria and actinomycetes isolation, extra soil environment was used with an add of gelosis. Later, special environment of Gause and Topping were used. For mold isolation, Martin environment was used mixed with Bengal pink and chloramphenicol. In certain herbicide substances, at a specific concentration, we noticed the appearance of algae. From these substances two dilution suspensions were prepared ( $10^{-1}$  and  $10^{-2}$ ), which were inoculated (1 ml) in Petri dishes (two repetitions), in a special environment for algae.

In order to create conditions similar to those found in the herbicide substances, before the distribution of culture medium, we placed in each Petri plate 0.3 ml of suspense herbicides, fit for inoculated dilution. The plates were covered with a glass vessel, exposed to the light and maintained at laboratory temperature. Also, at a concentration of 10 µg / ml a mold developed, which was placed in Petri plates, on a Martin environment with a Bengal pink solution (1:3000) and a streptomycin solution (1%). To the culture medium in every Petri plate an addition of 1 ml Chlorsulfuron herbicide solution has been added (mold medium). The plates were incubated at a temperature of 28°C.

## 3. Results and Discussion

After an incubation period of 1-7 days the most resistant microbial species have been highlighted from the three ecophysiological groups in the presence of the three tested substances (Table 1).

**Table 1.** Prokaryotes and eukaryotes resistant to the presence of sulphonylurea substances

Phytopharmaceutical Substance	Dose (g/ ha)	Resistant Prokaryotes and Eukaryotes				
		Bacteria (lab, field)	Actinomycetes (lab, field)	Micromycetes (lab, field)		
Glean	20	<i>Bacillus megaterium</i>	<i>S. griseus</i>	<i>Penicillium Rhizopus</i>		
	40		<i>S. albus, S. griseus</i>			
	100					
Grodyl	60		<i>S. griseus, S. aureus, S. albosporeus</i>			
	120					
	300					
Harmony	60					
	120					
	300					
Martor			<i>S. albus, S. aureus, S. griseus</i>			

Due to observation and experimental data (table 1) we established the following:

- *Bacillus megaterium* are a well – known colony, like white points, having a paste consistency;
- In most of treated substances, including the witness substance, *Streptomyces* is developed, especially the type of *S. griseus* and *S. albus*;
- From the identified varieties, there are two types of high level biodegradative capacity.

After 12 months of preparation in Chlorsulfuron, Thiphensulfuron and Amidosulfuron erbicides substances, microorganisms have been developed, which are resistant to these phytopharmaceutic substances (Table 2).

**Table 2.** Eukaryotic microorganisms resistant to suspense-herbicides

Phytopharmaceutical Substance	Concentration (µg/ml)	Resistant Eukaryotes	
		Algae	Molds
Glean	0.5	<i>Coccomyxa lacustris</i>	-
	10	-	unidentified
Grodyl	1	<i>Coccomyxa lacustris</i>	-
Harmony	1	<i>Coccomyxa lacustris</i>	-
	0.1		

After 3 weeks from the inoculation dilutions, native preparations were made also based on microscopic examination. Algae have been identified here. *Coccomyxa lacustris* can be found in dilution herbicides, which is part of the *Chlorococcales* group, from the *Radiococcaceae* family, it has ellipsoidal, oval cells, arranged in an irregular group or solitary, placed in a gelatinous sheath among the orbicular colon.

It may be irregular, more consistent, with a well-defined outline, parietal chloroplast and narrower. Cell size is between 5-7x23um. It is a planktonic species, widespread, but rarely reported.

A gray-brown-blackish mold has been found in the Chlorsulfuron substance (10 mg/ml). It has a glomerular shape (like a ball) and is located in the suspense column of herbicides.

Microscopic studies have been performed on the mold found in the herbicide dilution. Mold resistant to Chlorsulfuron was not identified.

#### 4. Conclusion

Among the most resistant bacteria species, at tested herbicides, isolated, both in field conditions and under controlled conditions, it is called *Bacillus megaterium*.

The most resistant species, like the *Streptomyces*, in most of tested herbicide doses, in field and laboratory are as follows: *S. griseus*, *S. albus*, *S. aureus* and *S. albosporeus*.

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