ABSTRACT

Studying the ecological risk of the herbicides used in modern agriculture is very important for environment protection from pollution. Therefore investigations of herbicide effect on important for soil fertility and plant nutrition microorganisms is actual and necessary. An ecological assessment of two herbicides (gardian and pivot) was done by measuring the bacterial growth of non-symbiotic nitrogen fixing bacteria - Azotobacter chroococcum 18 and two strains of Azospirillum brasilense - Sp. 7 and Sp. 107. Inhibition of bacterial growth is observed and it depends on the herbicide used, its concentration and the bacterial strain. Three rates of herbicides were studied. Gardian inhibition on the bacterial growth varies from 22% to 75%. The decrease of the size of bacterial colonies of A. brasilense Sp. 7, when the highest concentration is used is 75%; in case of A. brasilense Sp. 107 - it is 50%. In case of pivot application A. brasilense Sp. 107 is more resistant again (inhibition varies from 17% to 50%); for A. brasilense Sp. 7 it is respectively 33% and 56%. In case of A. chroococcum the inhibition of the highest concentration of gardian is 56% and of pivot - 50%. The results show that gardian is more toxic than pivot as concerning the growth of the bacterial species studied.

Keywords: gardian, herbicide, non-symbiotic nitrogen fixing bacteria, pivot

Introduction

Herbicide application in the intensive agriculture leads to a soil pollution risk, which includes a negative side effect on soil microflora. Therefore investigations of herbicide impact on important for soil fertility and plant nutrition microorganisms are actual and necessary (3,4). Studies of herbicide influence on microorganisms with environmental importance like azotobacter and other nitrogen fixing bacteria in vitro are less in number (5,6).

Investigations of gardian and pivot effect on the free living non-symbiotic nitrogen fixing bacteria Azotobacter chroococcum and the associative nitrogen fixer Azospirillum brasilense will give an information about the ecological assessment of these herbicides.

Materials and methods

The herbicide gardian / acetamide – 2 chloro – N – ethoxymethyl-N-(R-ethyl-6-methylphenyl) /, product of Monsanto (USA), was studied. A normal rate (usually used in agricultural practice) - 300 ml / da and two rates - increased 2,5 and reduced 2,5 times compared to the normal rate (respectively 750 and 120 ml / da) were examined. The studying of these two rates differing from the normal one, was done in order to establish the pesticide effect in extremal conditions – overdosage and reduced herbicide concentration in soil due to its decomposition. Gardian LD50 is 2,983 mg for rats.

The herbicide pivot / imidazolinone 5 – ethyl – 2 –(4 – isopropyl – 4 –methyl-5-oxo-2 imidazolin-2- yl) nicotinic acid /, product of Cyanamide (USA), was also studied in a normal rate of 120 ml / da and in increased and decreased doses, respectively - 300 ml / da and 48 ml / da. Pivot LD50 is more than 5000 mg for rats.

The microbiological risk assessment of gardian and pivot is done by measuring the bacterial growth of Azotobacter chroococcum 18 and of two strains of Azospirillum brasilense - Sp.7 and Sp.107 in vitro by the method of Bakalivanov (2).

Results and Discussion

The results show that both studied herbicides inhibit bacterial growth of the studied strains. Inhibition depends on the herbicide used, its dose and the bacterial species. The decrease of the size of bacterial colonies varies from 22% to 56% for gardian / Table 1 / and from 20% to 50% for pivot /
Table 2 / as concerning Azotobacter chroococcum 18. In case of Azospirillum brasilense the results show an inhibition, varying from 25% to 75% for gardian and from 17% to 56% for pivot. In case of both herbicides the inhibition of bacterial growth is proportional to the dose used.

**TABLE 1.**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Gardian concentration ml/da</th>
<th>Bacterial colony mm</th>
<th>LSD mm</th>
<th>Level of significance %</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azotobacter chroococcum 18</td>
<td>Control</td>
<td>120</td>
<td>7.0</td>
<td>1.56</td>
<td>0.1</td>
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<tr>
<td></td>
<td></td>
<td>300</td>
<td>6.0</td>
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<tr>
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<td></td>
<td>750</td>
<td>4.0</td>
<td>1.56</td>
<td>0.1</td>
</tr>
<tr>
<td>Azospirillum brasilense Sp. 7</td>
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<td>120</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td></td>
<td>300</td>
<td>2.5</td>
<td>0.78</td>
<td>0.1</td>
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<td></td>
<td></td>
<td>750</td>
<td>2.0</td>
<td>0.78</td>
<td>0.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Azospirillum brasilense Sp. 107</td>
<td>Control</td>
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<td></td>
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<td>0.78</td>
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<td>750</td>
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</table>

* control – without herbicide

**TABLE 2.**

<table>
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<tr>
<th>Bacterial species</th>
<th>Pivot concentration ml/da</th>
<th>Bacterial colony mm</th>
<th>LSD Mm</th>
<th>Level of significance %</th>
<th>Inhibition</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>120</td>
<td>8.0</td>
<td>1.10</td>
<td>0.1</td>
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<td>300</td>
<td>6.5</td>
<td>1.35</td>
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<td>5.0</td>
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<tr>
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</table>

A. chroococcum 18 is more resistant to both herbicides than A. brasilense Sp.7. A. brasilense Sp.107 is more resistant to both herbicides than A. brasilense Sp.7. This is very important for us because A. brasilense Sp.107 is an effective inoculant of wheat and other crops in our pot and field experiments (1). The data show that pivot has slightly weaker inhibitory growth effect than guardian (except in two cases).

Obviously, the inhibition depends as well as on the herbicide used and on the species and the strain studied. The greater resistance of azotobacter may be explained by the fact that it possesses a capsule, making herbicide penetration into the bacterial cell more difficult (7).
Conclusions

Gardian and pivot herbicides inhibit the growth of the non-symbiotic nitrogen fixing bacteria *Azotobacter chroococcum* and *Azospirillum brasilense*. Gardian is more toxic than pivot.

The inhibition depends on the herbicide used and on the bacterial species. The decrease of the size of bacterial colonies varies from 22% to 56% for gardian and from 20% to 50% for pivot as concerning *Azotobacter*. In case of *Azospirillum* the results show inhibition from 25% to 75% for gardian and 17% - 56% for pivot.

*A.chroococcum* 18 is more resistant to both herbicides than *A.brasilense* Sp.7.

*A.brasilense* Sp.107 is the more resistant *Azospirillum* strain

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REFERENCES