Biological bacterial herbicides solution based on pseudomonas

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Abstract. One of the problems of organic farming is weed control. There are some groups herbicides of biological nature today - fungal, bacterial, viral and some phyto toxins. The strain Pseudomonas syringae 564 by Danylo Zabolotny Institute of Microbiology and Virology NAS of Ukraine showed a herbicide’s effect against a number of monocotyledonous and dicotyledonous weeds.

1. Introduction
Microbial preparation of herbicide is defined as bioherbicides that may control the weed. Bioherbicides can be divided into 4 groups:

- Fungus (mycoherbicides);
- Bacterial;
- Viruses group;
- Natural compounds with herbicides effect.

Biological control of weeds using bacteria has several advantages over the use of fungal:

- Faster growth;
- Relatively simple requirements for reproduction;
- High suitability for genetic modification by mutagenesis or gene transfer [1].

There are several examples of the use of bacteria against weeds, mainly Pseudomonas fluorescens and Xanthomonas campestris but not Pseudomonas syringae. For example, strain Pseudomonas fluorescens - WH6, introduced into the root zone, inhibited the germination of 21 species of monocotyledons and 8 species of 2-lobed species, with the exception of corn [2].

Pseudomonas fluorescens strain D7, originally isolated from the rhizospheres of winter wheat (Triticum aestivum) and downy brome (Bromus tectorum) in Western Canada, has been observed to selectively inhibit growth and germination of a number of grassy weeds, most notably downy brome (Bromus tectorum L.) [3].

The effects of bioherbicidal activity of the bacterium Xanthomonas campestris against Conyza canadensis is known also [4]. Rosette leaf-stage plants were more susceptible than older plants, and increasing inoculum from $10^7$ to $10^9$ cells/mL caused significantly greater plant mortality and biomass reduction of plants in both the rosette and bolting growth stages.
However using bacteria against weeds has one problem - bacteria can’t enter in weeds plants, only through holes and wounds [5]. This disadvantage can be overcome by providing entrance wounds or using surfactants - penetrantes. The aim of our investigations - was to identify perspective bacterial strains and their concentration so that penetrants could be added to them and future solutions of biological herbicides could be prepared.

2. Materials and methods
The bioherbicide was applied to weeds that appeared on early cabbage plants in. Cabbage seedlings were planted on April 20 in phases of 5-6 true leaves. The soil in the Kyiv area is low-humus podzolic black soil. The area of the experimental plot is 5.6 m\(^2\), the repetition is 3 times. To prevent fusarium wilt and root rot under the roots of plants, the solution "Trichoderma" in grain form, by watering with a watering can. In June, the appearance of aphids was noticed on the plants. Against it we use spread - treatment with the biological product "verticillin" (Verticillium lecania). Weeds were not removed by loosening the row spacing’s, using only injections with a suspension of the bioherbicide.

Bacterial suspension for injection with a Pseudomonas syringae was prepared on a turbidity scale test tubes (McFarland standard test). Small drops of the suspension were applied to the lower surface of the leaf blade with a sterile Pasteur pipette, after which light injections were made through the drop with a thin sterile needle. Plants and leaves that were injected were marked with twine, tying it around. To find out the possibility of bacteria attacking the vascular system, a drop of the bacterial suspension was applied to the leaf petiole and the central vein of the leaf blade of Convolvulus arvensis, after which an injection was made through the applied drop according to the method of Beltyukova K. [6].

The ocular score of the prevalence of the disease on weed leaves was also determined in accordance with a 4-point scale also [7]:

- 0 - healthy plants;
- 1 - weak damage to an organ or plant;
- 2 - moderate damage, no severely damaged organs;
- 3 - severe organ damage and plant death.

The time of appearance of the first symptoms of the disease was determined. After 20 days, transverse sections of leaf petioles were made to determine vascular lesion of weeds. The intensity of the spread of the disease was evaluated by the area of spread of the symptoms of the disease on the leaves of the weed. Mathematical processing of the results was performed by the method of variation statistics.

3. Results and Discussion
The first shoots of weeds of a group of perennial and late spring crops appeared 2 weeks after planting the cabbages seedlings. Rosette leaf-stage plants were more susceptible than older plants [3], However, it is difficult to insert the needle at this time due to the small size of the weeds. Therefore, we introduced a bacterial suspension in the 3-4 leaf phase in weeds.

The first symptoms of damage to Convolvulus arvensis plants were observed 7-8 days after infection with a bacterial suspension. The intensity of the development of the disease can be characterized by a point of 1 - weak damage to the plant organ. On day 14, in the variant with a concentration of 4 McF, death to 50% of the sheet surface was observed, while in the variant with a half concentration (2 McF), up to 10%. On day 14, in the variant with a concentration of 4 McF, death to 90% of the sheet surface was observed, while in the variant with a half concentration (2 McF), up to 20% (figures 1 and 2). However, the growth of the weed continued, although some of its leaves were rotting. In the future, his complete death was not registration in our investigations.

The degree of weed infestation in the experimental plots increased by the harvesting period to 6-7 weed plants per 1 m\(^2\). Some of its were damaged with bacterial’s rot. Obviously, with such a number of weeds, it is necessary to test not the injections of the bacterial suspension, but the penetrants by
spraying [8-10], since the optimal concentration has already been determined. Also it would be interesting the introduction of a solution of a bacterial suspension into the soil zone where it could suppress weed plants.

It must be said that on other weeds, the concentration of the bacterial suspension - 4 McF was more effective than 2 McF (figures 3 and 4).

4. Conclusion
It can be concluded that the use of bacterial suspension strain Pseudomonas syringae 564 concentration 4 McF of turbidity scale test tubes can serve as material for biological herbicides and requires further development with the addition of penetrants. Also it would be interesting the introduction of a solution of a bacterial suspension into the soil zone where it could suppress weed plants.

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