Biologically active microorganisms for inhibition *Ambrosia artemisiifolia* L

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**Abstract.** The paper presents data on the evaluation of a number of microorganisms for biocontrol of the quarantine weed, *Ambrosia artemisiifolia* L. (common ragweed). Relatively high herbicidal efficacy (50% of dead seedlings of common ragweed) of multicomponent formulations based on a strain 32.85 of the necrotrophic phytopathogenic fungus *Stagonosporopsis heliopsidis* and a strain 4 of the phototrophic cyanobacteria *Nostoc sphaeroides* was experimentally demonstrated in laboratory conditions. This was twice higher compared to treatment with one microorganism. The emulsion formulation based on 0.5% refined vegetable oil + 1.0 % lecithin + 0.1% Tween 80 and 4% chopped mycelium of *S. heliopsidis* 32.85 was evaluated against *A. artemisiifolia* in the field conditions of Crimea foothills. The damaged leaf surface area was about 18% 2 weeks post treatment of the weed.

1. Introduction

*Ambrosia artemisiifolia* L. (common ragweed) is one of the dangerous weeds in the South of Russia, causing damage to crops, posing a threat to human health, and resulting in allergic reactions. Currently, the search for an alternative to chemical herbicides, which must be safe for ecosystems, biological preparation, as well as the development of organic methods for protecting agricultural lands from weeds are a priority. We focus on the ecologization of agroecosystems. The use of different phototrophic cyanobacteria and heterotrophic microorganisms as bioherbicidal effectors for the development of microbial preparations are relevant and ecologically friendly. The use of phytopathogenic fungi as mycoherbicides is one of the directions of the biological method of weed control [1, 2]. Phytopathogenic fungi are used as the basis of mycoherbicides to control the number of weeds in agroecosystems [3, 4].

Currently, 4718 species of cyanobacteria are described [5], more than 4000 phototrophic strains of cyanobacteria are studied, and more than 1000 secondary metabolites are investigated [6, 7] with antiviral, antibacterial, fungicidal, algicidal, antitumor actions, etc.

This will allow us to make a real contribution to reducing the pesticide load, as well as to controlling quarantine plants, improving social and economic development of the region according to the priority areas of the national economy of Russia.

The purpose of this study was to find biologically active microorganisms able to inhibit *A. artemisiifolia* L. in the conditions of the foothills of the Crimea.
2. Materials and methods

Strains of cyanobacteria *Nostoc linckia* 144, *N. sphaeroides* 4 and the heterotrophic strain *Bacillus sp.* 3С11 from the Crimean Collection of Microorganisms of Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea" (http://www.ckp-rf.ru/usu/507484/), strain *Nostoc calcicola* ACSSI 82 from the Algal Collection of Soil Science Institute (ACSSI) of the Institute of Physicochemical and Biological Problems of Soil Science RAS (http://acssi.org/index.php/catalogue), strain of micromycete *Stagonosporopsis heliopsidis* 32.85 from Collection of Federal State Budget Scientific Institution "All-Russian Research Institute for Plant Protection" of "State Collection of Microorganisms Pathogenic to Plants and their Pests" (http://www.ckp-rf.ru/usu/200616/) were used in the study. The research was carried out in 2019.

The strains of cyanobacteria were cultured in the recommended for the cultivation of cyanobacteria liquid mineral medium [8] in the climatic chamber at temperatures of +23-25°C, the photoperiod was 12 h. Cyanobacteric homogenate were based on different strains: *N. sphaeroides* 4 (СF144), *N. linckia* 144 (СF144), *N. calcicola* ACSSI 82 (СF82), *N. linckia* 144 (СF144). The load in homogenate was 0.1х10-3 mg of absolutely dry cell mass/ml. Homogenized cyanobacterial form was obtained by mixing disperse systems with liquid medium with fast-spinning rotor homogenizer. The filtrates were prepared from homogenized form and were used for treatment of weeds. Heterotrophic strain *Bacillus sp.* 3С11 was used as a bacterial suspension with an inoculation load of 10^6 cells/ml.

The strain *S. heliopsidis* 32.85 was cultured on soybean medium on a rocking chair for 4 days at 220 rotations per minute. The mycelium was separated from the culture liquid and crushed using a laboratory blender for 40 seconds. The crushed mycelium was used to prepare mycopreparative forms (MF, 4 g of mycelium per 100 ml) and then diluted with water or cyanobacterial filtrate.

The effect of inhibition with preparative forms was studied on *A. artemisiifolia* in a laboratory experiment on southern chernozem. Plants were grown in 200 ml plastic cups in the climatic chamber at temperatures of +23-25°C, the photoperiod was 10 h. Each plant in the phase of the four true leaves was sprayed with 10 ml of suspension. Such biometric indicators as plant height, vegetative and root phytomass, and the percentage of plant death were taken into account in the experiment. The experiment design was as following 1) control – water; 2) PF; 3) PF + H_2O (1:100); 4) PF + H_2O (10:100; 5) PF + CF_4 (1:100); 6) PF + CF_144 (1:100); 7) CF_4; 8) CF_144; 9) *Bacillus sp.* 3С11.

The effectiveness of mycoherbicides in the field is lower than that of chemical herbicides [9]. The propagules of fungi require a fairly long presence of moisture on the plant to germinate and infect plant tissues. It is possible to reduce the dependence of fungi from the duration of the dew period and increase the infectivity of propagules by optimizing the formulations by introducing moisture-retaining additives, adhesives, surfactants and nutrients [10, 11]. Biologically active and surface-active substances: Sylwett, Tween 80, glycerol, refined vegetable oil, and lecithin were added to the preparative forms to enhance the herbicidal effect.

We evaluated the following preparative compositions:

1. Control – liquid solution 0.1 % Sylwett.
2. Control – liquid solution 0.1% Tween 80.
3. Control – liquid solution 0.1% Tween 80 + 0.5% glycerol.
4. Control – emulsion based on 0.5% refined vegetable oil + 1% lecithin + 0.1% Tween 80.
5 – 8. Mycelium of *S. heliopsidis* 32.85 (m) was added to each of the listed control compositions in the amount of 40 g/l.

Herbicidal efficiency of the preparative compositions was studied in a field experiment on common ragweed using a method [12] on the southern chernozem in foothill climate zone of the Crimea. Agrochemical analysis of the soil was carried out according to generally accepted methods in the Laboratory of Agrochemical Research (structural unit of the Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea").

The field experiment was conducted in the phytocenosis *A. artemisiifolia* at abandoned land plot (Simferopol region, GPS point N44°99.511′, E34°07.080″). The area of the accounting plot is 2 m². Climate of the foothill zone is moderately continental. Soil is heavy loam carbonate chernozem with
2.0-3.4 % of humus in the arable horizon. The annual precipitation is 450-550 mm with its maximum (68 mm) in June. The number of *A. artemisiifolia* plants was counted before bioherbicides application. The treatments with the bioherbicidal formulations were carried out with a manual sprayer on ragweed plants in the stem elongation phase in the morning hours. The air temperature was 19-20°C, wind speed – 1-2 m/s. Counting of the death weeds was carried out at fixed areas after 5 and 8 days after making bioherbicidal treatment.

3. Results and Discussion

Strains of phototrophic and heterotrophic bacteria were evaluated for their ability to inhibit *Ambrosia artemisiifolia* L. plants in a laboratory experiment. In control, bacterization with the strain *S. heliopsidis* 32.85 resulted in the death of 25% of plants (Table 1).

| No | Variant of the experiment | Plant height, cm X ± SE | Plant above-ground, g X ± SE | Plant root mass, g X ± SE | % of dead plants |
|----|--------------------------|-------------------------|-------------------------------|--------------------------|-----------------|
| 1  | H2O                      | 4.68 ± 0.31             | 0.11 ± 0.01                   | 0.13 ± 0.01              | 0               |
| 2  | MF                       | 5.57 ± 0.58             | 0.13 ± 0.02                   | 0.12 ± 0.02              | 25              |
| 3  | MF+H2O (1:100)           | 5.87 ± 0.47             | 0.12 ± 0.01                   | 0.08 ± 0.03              | 25              |
| 4  | MF+H2O (10:100)          | 5.18 ± 0.65             | 0.12 ± 0.04                   | 0.12 ± 0.02              | 0               |
| 5  | MF+CF4 (1:100)           | 7.00 ± 0.50             | 0.12 ± 0.01                   | 0.13 ± 0.03              | 50              |
| 6  | MF+CF144 (1:100)         | 5.00 ± 0.25             | 0.10 ± 0.01                   | 0.13 ± 0.01              | 25              |
| 7  | CF4                      | 5.67 ± 0.24             | 0.13 ± 0.02                   | 0.14 ± 0.04              | 25              |
| 8  | CF144                    | 6.05 ± 0.73             | 0.13 ± 0.01                   | 0.14 ± 0.01              | 0               |
| 9  | *Bacillus sp* 3C11       | 4.45 ± 0.53             | 0.07 ± 0.02                   | 0.10 ± 0.03              | 0               |

Notes: MF – mycopreparative form based on *S. heliopsidis* 32.85; CF4/144 – cyanobacterial filtrates based on *Nostoc sphaeroides* 4 / *Nostoc linckia* 144.

The treatment of plants with the preparation form of this strain in dilution with water 1: 100 and treatment in the same dilution with a filtrate based on *N. linckia* 144, and mono-treatment with a filtrate based on *N. sphaeroides* 4 showed a similar effect. Treatment with mycopreparative form of *S. heliopsidis* 32.85 and the filtrate of *N. sphaeroides* 4 led to 50% death of *A. artemisiifolia* in laboratory conditions. We can make an assumption about the synergism of the components of mycelial and cyanobacterial preparative forms. In other variants of the experiment, no dead plants were found.

The effectiveness of *A. artemisiifolia* treatment with compositions based on mycelium of *S. heliopsidis* 32.85 and surfactants was evaluated in the field experiment. The ragweed density was 23-31 plants/m². Yellowing of the leaves was established after five days of observation in the treatment with refined vegetable oil + lecithin + Tween 80 + *S. heliopsidis* (Figure 1, a). There was no significant difference in the state of the plants in the other variants.
Figure 1. Reaction of *A. artemisiifolia* after with compositions based on the vegetable oil + lecithin + Tween 80 + mycelium of strain *S. heliopsidis* 32.85: a – fifth day; b – eighth day; circles indicate yellowing (a), necrosis (b).

In the control treatment with adjuvants and surface-active substances along did not have any effect on the weed after 8 days of observation (Table 2). Considerable leaf damage was achieved with the emulsion-based formulation of *S. heliopsidis* 32.85. Leaf necrosis and dead plants were observed on the variant with compositions based on the vegetable oil + lecithin + Tween 80 + mycelium of strain *S. heliopsidis* 32.85 (Figure 1, b).

Table 2. Efficiency of treatment of *A. artemisiifolia* with compositions based on the mycelium of the *S. heliopsidis* 32.85 and biologically active and surface-active substances (field experiment in the foothills of the Crimea, average on the 8th day of the experiment)

| No | Variant of the experiment | Number of plants before treatment, pieces/m² X ± SD | Incidence of infection of plants, % X ± SD |
|----|---------------------------|---------------------------------------------------|------------------------------------------|
| 1  | Sylwett                   | 27.5±3.54                                         | 0                                        |
| 2  | Tween 80                 | 31.0±4.24                                         | 0                                        |
| 3  | Sylwett +Tween 80        | 23.0±4.24                                         | 0                                        |
| 4  | RVO + Lecithin + Tween 80| 33.0±1.41                                         | 0                                        |
| 5  | Sylwett + m              | 31.0±1.41                                         | 4.50±2.12                               |
| 6  | Tween 80 + m             | 30.5±2.12                                         | 3.25±0.21                               |
| 7  | Sylwett +Tween 80 + m    | 28.5±3.54                                         | 7.35±5.87                               |
| 8  | RVO + Lecithin + Tween 80 + m | 31.0±1.41 | 17.70±2.97                             |

Notes: RVO – refined vegetable oil, m – mycelium of strain *S. heliopsidis* 32.85.

Treatment with an emulsion based on 0.5% refined vegetable oil + 1% lecithin + 0.1% Tween 80 and *S. heliopsidis* provided the maximum reliable incidence of plant lesions (17.7%). The effectiveness of this composition was 2.4 – 5.4 times higher compared to other bioherbicidal formulations.

4. Conclusion

Thus, preparation based on *S. heliopsidis* 32.85 and the cyanobacterium *N. sphaeroides* 4 provided a reliable death rate of *A. artemisiifolia* plants at the level of 50%, which is twice as effective in comparison with mono-treatment. This fact was experimentally established in laboratory conditions.

Formulations based on the adjuvants and surface-active substances jointly with mycelium of *S. heliopsidis* 32.85 were evaluated against common ragweed in the field conditions of the Crimea foothills for the first time. The fungus formulated as an emulsion provided considerable damage of the weed at the level of 17.7%. This is the ecologically safe basis for the development of biotechnology for controlling such quarantine weed as *A. artemisiifolia*.
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