Microbiological analysis of agropal soil with potato plantings

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Abstract. In this article, the soils of experimental beds of arable land with potatoes of the Tittyakh plot are studied. A general microbiological analysis of the selected soils—determination of the main ecological and trophic groups of microorganisms, as well as identification of the presence of phytopathogenic fungi contributing to potato spoilage—was carried out. It has been established that the used pesticides (herbicides) reduce the total number of microorganisms in the arable land with potatoes by 3 times and have different effectiveness against pathogenic fungi for potatoes. It was revealed that the soil at the Tittyakh site is infected with the most dangerous fungi for potatoes from the genera Fusarium, Trichoderma, Alternaria.

1. Introduction
Potato cultivation in the Republic of Sakha (Yakutia) is complicated by the presence of a number of negative natural factors: late spring and early autumn frosts, and therefore a short growing season, in June - August, heat, soil drought, dry winds, as well as sharp changes in air temperature from day to night are not uncommon. Permafrost soils with low nitrogen content and alkaline reaction of the environment are common here, there is a widespread and harmfulness of some diseases, especially rhizoctoniosis, scab, wet and dry rot of tubers. However, despite these negative conditions, potatoes have been grown here for about 200 years [1]. At the same time, there is an acute problem of diseases of potato. In Yakutia, the study of the prevalence and harmfulness of potato diseases has not been carried out enough until recently.

The objects of research of this scientific work, along with bacteria, are also microscopic fungi. From mushrooms, the death of cultivated plants and the spoilage of food products are caused by imperfect fungi (Deuteromycetes). Representatives of the genera Fusarium, Trichoderma, Alternaria are also widespread in nature, which are found on plant residues, fruits, seeds and in the soil.

The purpose of this study was to determine the total number of microorganisms in arable soil with potatoes and phytopathogenic fungi for potatoes in soil treated and not treated with herbicides.

2. Materials and methods
Soil and microbiological studies of the soils of arable land with potatoes were carried out. In the course of soil research, we used well known soil methods, such as profile-genetic [2] and comparative-analytical [3], and the physico-chemical properties of the soil were determined according to generally accepted methods in soil science [4].

Soil samples for microbiological analysis were selected once on 01.08.2018. The weather was hot, the air temperature ranged from +30 to +32°C. The samples were taken from the soil of the Tittyakh
site located on the floodplain terrace of the Lena River, 1.5 km from the village of Oi (Nemyugyuntsy) of the Khangalassky ulus and 72 km from Yakutsk. The arable land was not irrigated, the soil was dry. Sampling was carried out according to the standard procedure [5]. Soil samples for microbiological studies were taken from 6 potato ridges in layers from depths of 0-20 and 20-40 cm. The method of sampling, storage and delivery of samples excluded the possibility of their thawing and infection by extraneous microorganisms.

In the control version, unlike the experimental ones, herbicides were not used. In the experimental versions, different herbicides were used, their composition and quantity, the timing of application and the method are unknown to us.

The number of microorganisms was determined by seeding on dense nutrient media. The number of bacteria using organic nitrogen was taken into account on MPA, bacteria and actinomycetes using mineral nitrogen - on KAA, oligonitrophilic bacteria - on Ashby medium [5]. The number of fungi was taken into account on the acidified medium of Chapek [6]. The seeded Petri dishes were incubated at 30°C. Colonies of bacteria were taken into account for 3-4 days, fungi - for 7-10 days (on 5 parallel cups based on 1 g of absolutely dry soil). The morphology of the cultures was studied by light microscopy using a microscope "Biolam R-15" (magnification 1250).

3. Results and Discussion

To describe and characterize the studied soil, a soil section of 5th-04 was laid on the arable land of the Tittyakh site. The coordinates of the section: N - 61°31'. 211's.w., E - 129°10.659's.d., H - 110.9 m. Morphological structure of the profile: Aa(0-30)–ABca(30-42)–BCca(42-68)–Cca(68-137cm). Soil: permafrost agropalic.

**Table 1. Properties of the studied permafrost pale yellow soil.**

| Horizon | Depth, cm | pH_{H2O} | Humus, % | Sum of salts, % | CO₂ Carbonates, % | Exchange cations, resin(eq)/kg of soil | Fraction content, % |
|---------|-----------|----------|----------|-----------------|------------------|----------------------------------------|---------------------|
|         |           |          |          |                 |                  | Ca^{2+} | Mg^{2+} | Na^{+} | <0.001 ml | <0.01 ml |
| Aa      | 0-10      | 8.0      | 2.1      | -               | -                | 17.0     | 6.1     | 1.6     | 5.2        | 15.2       |
| ABca    | 20-30     | 8.3      | 2.2      | 0.106           | 5.2              | 17.3     | 6.9     | 1.1     | 5.0        | 15.2       |
| BCca    | 50-60     | 8.4      | 0.8      | 0.105           | 10.4             | 18.9     | 7.3     | 1.2     | 5.4        | 16.4       |
| Cca     | 90-100    | 8.7      | 0.5      | 0.089           | 10.5             | 29.4     | 8.4     | 1.4     | 8.6        | 19.3       |

Morphological and physico-chemical properties of the studied sample is pale-yellow that allowed us to state that this soil has undergone a significant agrogenic transformation in the process of long-term agricultural development and irrigated agriculture. So pH_{H2O} changes from slightly alkaline to hor. Aa to alkaline in the lower part of the soil profile [7], the humus content in the humus horizons of Aa and ABca, according to the well-known scale [8], is defined as low. Also, this soil is characterized as unsalted in accordance with the gradations [9], when the amount of salts is 0.089-0.106%, of light sandy loam granulometric composition [10], as well as a soil-absorbing complex saturated with exchange bases (table 1).

According to physico-chemical properties, the studied agropal soil turned out to be slightly alkaline, unsalted and low humus. The alkalinity increased with depth, and the humus content decreased. The temperature in the profile of this soil decreased sharply from 20.6°C in the humus horizon to 8.7°C in the lower mineral. The soil was very dry, so its humidity was low (table 2).

The number and composition of the main groups of microorganisms of agropal soil. At the very beginning, we conducted a microbiological analysis of the microflora of soil samples from experimental beds of arable land on agropal soil. The results of this analysis are presented in table 2.

From the data presented, it can be seen that prokaryotic microorganisms or bacteria predominate in the control soil not treated with herbicides. Mycelial fungi (Eukaryotes), among them phytopathogenic
fungi, were found as little as 5.06 million CFU/g in the upper layer (5.1 x 10^{6}), up to 6.56 million CFU/g (6.6 x 10^{6}) in the lower soil layer.

**Table 2.** Total number of microorganisms in agropal soil.

| Option | Ridge number | Cipher | Depth, cm | Humidity % | Number of microorganisms, CFU/g |
|--------|--------------|--------|-----------|------------|---------------------------------|
|        |              |        |           |            | MPA                             |
|        |              |        |           |            | Ashby                           |
|        |              |        |           |            | KAA                             |
|        |              |        |           |            | Chapek                          |
| Control | 1            | B – 1  | 0-20      | 10.66      | 2.30x10^7                       |
|         |              |        | 20-40     | 16.57      | 1.07x10^7                       |
|         |              |        |           |            | 1.35x10^7                       |
|         |              |        |           |            | 5.05x10^6                       |
| 2       |              | B – 2  | 0-20      | 10.05      | 1.22x10^7                       |
|         |              |        | 20-40     | 15.32      | 9.17x10^6                       |
|         |              |        |           |            | 3.38x10^6                       |
|         |              |        |           |            | 1.36x10^7                       |
| 3       |              | B – 3  | 0-20      | 11.25      | 1.90x10^7                       |
|         |              |        | 20-40     | 18.18      | 1.94x10^6                       |
|         |              |        |           |            | 1.30x10^6                       |
|         |              |        |           |            | 1.59x10^7                       |
| Experience | 4          | B – 4  | 0-20      | 11.54      | 5.05x10^6                       |
|          |              |        | 20-40     | 13.95      | 5.15x10^6                       |
|          |              |        |           |            | 1.31x10^6                       |
|          |              |        |           |            | 7.46x10^6                       |
| 5       |              | B – 5  | 0-20      | 10.91      | 8.36x10^6                       |
|         |              |        | 20-40     | 15.15      | 3.20x10^6                       |
|         |              |        |           |            | 2.00x10^6                       |
|         |              |        |           |            | 3.45x10^6                       |
| 6       |              | B – 6  | 0-20      | 9.90       | 1.22x10^7                       |
|         |              |        | 20-40     | 18.69      | 2.80x10^6                       |
|         |              |        |           |            | 2.16x10^7                       |

In the control soil which was not treated with pesticides, ammonifying bacteria on MPA prevailed, their number in the upper layer was 2.30x10^{6} CFU/g of soil, while the number of oligonitrophilic bacteria on Ashby medium reached 1.07x10^{6} CFU/g of soil ASB.

Thus, on August 1, 2006, the total number of microorganisms in potato arable land was small. A comparison of the total number in the upper soil layer showed that oligonitrophilic bacteria predominate in the control soil, not treated with pesticides (table 2). Their number ranged from the minimum in the variant 4 - 280 thousand cells/g (2.8 x 10^{5}) to 107 million cells/g (1.07 x 10^{8}). In the experimental versions, the number of oligonitrophils was 3 orders of magnitude less than in the control. The influence of pesticides reduced the number of bacteria by three times. Next come the ammonifying bacteria and in third place the fungi. The most mycelial fungi were found in the experimental 6 variant of 21.64 million CFU/g (2.16x10^{7}).

In the lower layer of 20-40 cm, on the contrary, eukaryotes – fungi prevailed, their number was greatest in variant 4 - 3.45x10^{7} CFU/g and variant 5 - 2.37x10^{7} CFU/g, and in the control - 6.56x10^{6} CFU/g. An increase in the number of fungi at a depth of 20-40 cm shows that pesticides have not penetrated here, their effect is detected only in the upper 20 cm layer. Mycelial actinomycetes (KAA) – 3.38x10^{6} in variant 2 and 1.31x10^{6} CFU/g in variant 4 also dominated among the grown microorganisms.

The use of pesticides has also affected the number of mycelial fungi. The growth and reproduction of fungi were noticeably suppressed in the 3rd experimental version. Here, in the 20 cm layer, their number was the smallest and amounted to only 1.59 x 10^{5} cells/g of soil. In the lower layers, pathogenic fungi were present in noticeable quantities, especially in variant 4, 3.45 x 10^{5} CFU / g, which indicates a slight penetration of the poison into the soil or insufficiently effective sealing.

The comparatively smaller number of these microorganisms [11-12] may be related to the pH value of the medium, since most of them prefer a pH in the range of 6.5-7.5, and fungi reproduce well at an acidic pH value, or at a pH of 8-10.

Identification of phytopathogenic fungi in agropal soil. To determine the generic affiliation of fungi pathogenic to potatoes from different variants, seeding was done on dense nutrient media of MPA and Chapek. The results are shown in figure 1. From the experimental variants, we isolated representatives of the genera Fusarium, Trichoderma, Alternaria [13].
**Figure 1.** Mycelial fungi of genus Trichoderma (A, left) option 2 and Alternaria (A, right) option 4. Mycelial fungi of genus Fusarium (B), isolated from option 5, this arable land, depth 20–40 cm, 12 days, medium Chapek.

Fungi of the genus Trichoderma (figure 1A, left) appeared on the surface of the medium on acidified wort-agar after two to three days of incubation at 23-25°C, first white, then green-tinged areas with a loose patchy or felt surface formed by mycelium and a cluster of conidiophores. With time, they turn dark green [13-14].

Alternaria (figure 1A, right) are characterized by a peculiar structure of multicellular pear-shaped conidia connected by chains. Colonies are light, fluffy at first, then greenish-gray or olive-black, velvety or fleecy, often with a pronounced concentric zonality; sometimes from the very beginning they are sooty-black, in many cases the dark pigment diffuses into the medium [14].

For further identification of mushrooms, we set up a model experiment on fresh potatoes. A slice of potato was placed in test tubes, two volumes of distilled water were added and sterilized (1 atm.) [5].

Then, colonies of fungi isolated from different variants of potato arable land and control, grown on media: Chapek and MPA, were sown. The control was a potato slice on sterile distilled water and an option-control from the garden, without treatment with herbicides. After 15 and 30 days, a description was made and photographed. The results are shown in figure 2. Figure 2 shows an unchanged potato slice in clear water (A, in the center). Next to it is a bright yellow mycelial fungi of genus Fusarium, the pigment colored the liquid medium, option 4, depth 20-40 cm. In other test tubes, potato slices of varying degrees of decomposition, primary mycelium on top in the form of a film and turbidity of the medium as a result of butyric acid fermentation under the influence of anaerobic bacteria are visible.

The results of observation of the growth of mycelial fungi isolated from potato arable land on day 30 (figure 1B) showed that in the control variant with water (the first tube on the left), the slice did not change and the medium is transparent. In the control from the experience of option 1 without the use of pesticides, the soil contains a lot of fungi, but there are differences in soil layers. There are practically no fungi on the surface (0-20 cm), perhaps this is due to the dryness of the soil (2 test tubes on the left), the potato slice is not changed. In the 3rd test tube (figure 2 B), a very cloudy medium is visible, this is a control variant from the lower layer (20-40 cm), the slice is completely decomposed, which indicates the presence of species of genus Fusarium. In 4 and 5 test tubes, variants 2 and 5 are presented, respectively, a strong decomposition of the slice, a bright yellow pigment in the medium, all indicates the presence of genus Fusarium species. In other test tubes, except the last one, the medium is more transparent, the slice is poorly decomposed. In the last test tube, genus Fusarium is clearly present, this is the upper layer (0-20 cm) option 4. Since the pigment is a diagnostic feature for systematic units of genus Fusarium, it requires the establishment of standard terms for describing the pigment, as well as for measuring conidia. The pigment is most pronounced on the 30th day. Later, cultures are usually covered with secondary mycelium, masking the primary one, or the mycelium begins to fade, as a result of which it becomes uncharacteristic. Therefore, this period was set as the standard for the description of the pigment in the studied fusariums [15].
Figure 2. Test tubes with a slice of potato, sown (infected) with fungi isolated from potato arable land, on day 15 (A), on day 30 (B). Mycelial fungi of genus Trichoderma (C, test tube far right) and genus Fusarium (C, yellow in the center) on a slice of potato.

In order to show the growth of isolated fungi on potatoes, water was partially drained from some test tubes and the test tubes were cultivated in the light (figure 2B). Fungi of the genus Trichoderma after 2-3 days of incubation at 23-25°C, first white, then green-tinted areas with a loose patchy or felt surface formed by mycelium and a cluster of conidiophores appear on the surface of potatoes [14]. With age, they turn dark green. In the picture, the felt, bluish-green mycelium genus Trichoderma is clearly visible on an almost decomposed potato slice (in 4 test tubes), option 5, depth 0-20 cm. The results showed that the experimental potato beds of the Tittyakh plot were indeed infected with phytopathogenic microscopic fungi and treatment with herbicides suppressed the growth of not only fungi, but also bacteria.

4. Conclusion
Based on the research and analysis carried out, the following general conclusions can be drawn:

- The used pesticides reduce the total number of microorganisms in permafrost agro-field soil by 3 times. At the same time, the used pesticides have different effectiveness against pathogenic mushrooms for potatoes.
- The most effective inhibit the growth of mycelial fungi is the toxic chemical used in option 3. To combat pathogenic fungi, it is necessary to seal chemical reagents deeper and apply intensive watering after applying reagents.
- The agropal soil of the Tittyakh site is infected with the most dangerous fungi for potatoes from the genuses Fusarium, Trichoderma, Alternaria. To prevent and reduce the damage of potatoes with dry and black rot, careful handling of tubers during harvesting, transportation and storage is required, maintaining an optimal storage mode.

Acknowledgments
The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 0297-2021-0027, reg. No. AAAA-A21-121012190033-5).

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