The occurrence of antagonists microorganisms to phytopathogenic fungi in consideration of various tillages

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Abstract. The influence of different tillage methods (20-22 cm plowing) with applying nitrogen fertilizer and without it on the occurrence of antagonist microorganisms to phytopathogenic fungi Bipolaris sorokiniana was studied. Tillage with overturning soil (20-22 cm plowing) increased credibly (p<0.01) the frequency of occurrence of antagonist microorganisms in compare with No-till. The using nitrogen fertilizer (ammonium nitrate) didn’t affect the occurrence of antagonist microorganisms.

1. Introduction
The soil is the base for agriculture and a place, where almost all plants are growing. Widespread using fertilizers and pesticides has affected on soil quality and leaded to dangerous environmental consequences. In this regard the interest to creation of biological methods of combating phytopathogenic fungi based on the use of microorganisms antagonists of the pathogen was increased last years [1, 4, 5].

Contrariwise, tillage is crucially important for regulation activity and abundance of suppressive microflora. Harrowing and stirring of soil during plowing (with overturning soil) credibly intensify erobic microorganisms’ development [2, 3]. At the same time, spring wheat growing after green fallow (mustard phytomass was incorporated into the soil like organic fertilizer) stimulates the reproduction of antagonistic and saprotrophic microflora, as a result of which the survival of phytopathogens in the soil is reduced.

The goal of our research was estimate of occurrence of antagonist microorganisms to phytopathogenic fungi in consideration of tillage with overturning soil (20-22 cm plowing) and No-till with using nitrogen fertilizer and without it.

2. Methods and results
Objects of the research were the microbial community of soils, which are located in educational training farm of "Minderlinskoe", township Borsk, Sukhobuzim district of Krasnoyarsk Territory. Soil samples were taken from “Novosibirskaya 15” spring wheat grown after green fallow with overturning soil tillage (20-22 cm) and without tillage, with applying nitrogen fertilizer (ammonium nitrate) and without it, as well as from after wild perennial grasses (table 1). It was found, that soils with increased and high humus occupy the maximum arable land area (73.4 %). These soils are mainly leached chernozems in combination with ordinary chernozems. Leached chernozem characterized by a raised maintenance of humus (6.1-8.0%), a neutral reaction of the soil solution (pH - 6.1-7.0), particle size...
distribution was heavy loamy. In the topsoil of leached chernozem an increased content of mobile phosphorus (200-250 mg / kg of soil) and a very high exchange potassium of more than 150.1 mg / kg K2O were noted.

**Table 1. Design of experiment**

| Code | Previous crop | Nitrogen fertilizer | Plowing | Pesticides | The number of isolates |
|------|---------------|---------------------|---------|------------|-----------------------|
| Wheat + Plowing + Nitrogen fertilizer + Pesticides | Sidereal fallow | Yes | Yes | Yes | 30 |
| Wheat + Plowing + Pesticides | Sidereal fallow | No | Yes | Yes | 30 |
| Wheat + Nitrogen fertilizer + Pesticides | Sidereal fallow | Yes | No | Yes | 30 |
| Wheat + Pesticides | Sidereal fallow | No | No | Yes | 30 |
| Wild perennial grasses | No | No | No | No | 30 |

Test objects of the research were microscopic phytopathogenic fungi Bipolaris sorokiniana, which were taken from affected wheat organs (figure 1).

Isolation and cultivation of soil autochthonous bacteria was carried out on PDA (peptone enzymatic - 9.0, casein hydrolyzate enzymatic - 8.0, yeast extract - 3.0, sodium chloride - 5.0, sodium hydroorthophosphate - 2.0 + 0.5, pH (7.2±0.2). Antagonistic activity was inspected on the artificial nutrient medium of the timing of Saburo by the method of co-cultivation of macrocolonies. For this purpose, Bipolaris sorokiniana was inoculation on a solid medium in the center of the plate, bacteria were inoculation around the fungus at certain intervals. Inoculation was carried out in such a way that isolated colonies accessible to quantification were grown on the plate. Antagonist bacteria were determined by the zone of lack of growth of phytopathogenic fungus during of cultures enlargement (figure 2). Cultivation was carried out at 25°C for 7 days.
Statistical analysis was performed with the use of analysis of 2x2 contingency tables by the Fisher exact criterion (F-criterion) and analysis of contingency tables of arbitrary dimension according to the $\chi^2$ criterion ("chi-square").

![Figure 2. Inspection of antagonistic activity antagonist microorganisms to phytopathogenic fungi Bipolaris sorokiniana.](image)

Bacteria order Bacillales and Actinobacteria were dominated among antagonists (figure 3).

![Figure 3. Cell morphology autochthonous soil antagonist bacteria order Bacillales (left) and Actinobacteria (right), x100 lens, phase contrast, oil immersion.](image)

Microorganisms exhibiting antibiotic activity against phytopathogenic fungi Bipolaris sorokiniana were found in all studied communities.

A study of the influence of various methods of tillage with application of nitrogen fertilizer and without it showed that the largest number of antagonist microorganisms to fungi Bipolaris sorokiniana (16 clumps) was found wheat after green fallow with overturning soil tillage (plowing 20-22 cm) without nitrogen fertilizers. The applying nitrogen fertilizers reduces the amount of antagonist microorganisms (15 clumps). Least amount of antagonist microorganisms was founded under wheat after green falls No-till with applying fertilizers and without it (9 and 7 clumps) (figure 4).
Figure 4. The influence of overturning tillage and applying fertilizers on the number of antagonist microorganisms.

There are no statistically significant differences in the occurrence of antagonist microorganisms in the table by criteria $\chi^2$. There are statistically significant differences ($p<0.05$) between two options of study “wheat after green fallow with tillage without fertilizers” and “wheat after green fallow with tillage and applying fertilizers” in pairwise comparison by Fisher’s exact test for 2x2 tables.

Comparison research results in type of tillage showed statistically significant differences ($p<0.01$) between overturning soil tillage and No-till (by Fisher’s exact test for 2x2 tables). The effect of nitrogen fertilizer (ammonium nitrate) on the occurrence of antagonist microorganisms (the one-tailed P value equals 0.3855) not found (by Fisher’s exact test for 2x2 tables). As a result of research, overturning tillage (plowing 20-22 cm) credibly increased frequency of antagonists (practically in 2 times: 31 antagonists of 60 tested clumps) in compare with No-till (16 antagonists of 60 tested clumps).

References
[1] Iván Petatán-Sagahón, Miguel Angel Anducho-Reyes, Hilda Victoria Silva-Rojas, Ainhoa Arana-Cuenca, Alejandro Tellez-Jurado, Isabel Oyuki Cárdenas-Álvarez and Yuridia Mercado-Flores 2011 Isolation of Bacteria with Antifungal Activity against the Phytopathogenic Fungi Stenocarpella maydis and Stenocarpella macrinspora *International Journal of Molecular Sciences* **12**(9) 5522-37
[2] Pamela A., Harris Harry H., Schomberg Philip A., Banks Joel Giddens 1995 Burning, tillage and herbicide effects on the soil microflora in a wheat-soybean double-crop system *Soil Biology and Biochemistry* **27** (2) 153-6
[3] Shixiu Zhang, Qi Lia, Ying Lü, Xiaoming Sunac, Shuxia Jiab, Xiaoping Zhan, Wenju Lianga 2015 Conservation tillage positively influences the microflora and microfauna in the black soil of Northeast China *Soil and Tillage Research* **149** 46-52
[4] Trujillo-Tapia M N, Ramirez-Fuentes E 2016 Bio-fertilizer: an alternative to reduce chemical fertilizer in agriculture *Journal of Global Agriculture and Ecology* **4**(2) 99-103
[5] Zheng S, Chen B, Qiu X, Chen M, Ma Z and Yu X 2016 Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China *Chemosphere* **144** 1177–9